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Comparative Phytochemical Analysis of Different Market Samples of Asoka (*Saraca indica* Linn) in Kerala

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ABSTRACT

Asoka (*Saraca indica Linn*) is a rainforest tree belongs to the family Fabaceae. It is one of the extensively used medicinal plant in Ayurveda. Asoka or Ashoka is a Sanskrit words which means “without sorrow” or which that gives no grief. Now a days there are significant number of methods to authenticate crude drugs. A simple method like organoleptic characteristics may hold good to assess the genuinity of certain drugs but some may require highly sophisticated techniques too, based on the adulterants and similarity in the chemical constituents. So it is in the hands of the researchers to choose the right method suitable for the drug of interest which could match the reference standard. DNA fingerprinting, Gas Chromatography, High performance liquid chromatography are the most sophisticated methods which can assess the ingenuity very keenly, will help in the further studies. Different market samples of Asoka (*Saraca Indica* Linn) were analysed to screen the genuinity of the samples available in the market in the name of Asoka. All the samples were subjected for Prelimary Phytochemical analysis, HPTLC, ICPMS and Fluorescence spectroscopy. The comparative analysis with the genuine and the market samples was inferenced that all the samples were derived from different botanical sources.

INTRODUCTION

Asoka (*Saraca indica Linn*) is a rainforest tree belongs to the family Fabaceae[1]. It is one of the extensively used medicinal plants in Ayurveda. Asoka or Ashoka is a Sanskrit words which means "without sorrow" or which that gives no grief. In Vrksayurveda, it has been mentioned that Asoka is one among the *Mangalyavrksha,* that is *Pada Pancaka*[2]. The bark of Asoka has been used since time immemorial. It is one of the most significant Unani and Ayurvedic medicinal plants for the treatment of several feminine disorders especially in menorrhagia & gynaecological disorders. It has stimulating effect on endometrial and the ovarian tissue. It is used in many uterine diseases due to its strong hemostatic property and astringent effect on uterine muscles, and is called as "female tonic".

Manufacturers across the country use about 2,250 million tons of barks and flowers annually, and its need is increasing at the rate of 15 % annually. But the current conservation status of Asoka says that there is an immense decline in the population of this species, and it is becoming rarer in its natural habitat. It has been listed in the "Vulnerable" category by IUCN. Asoka has been named one of the 32 priority species by National Medicinal Plants Board [3]. Unscientific management practices and destructive harvesting process led Asoka into the priority species. Even considering all these factors interestingly Asoka bark is abundant in our markets. Because of destructive extraction and the absence of an organized cultivation program, the availability of crude drug is diminishing and this has resulted in the sale of adulterants. Due to more demand and less supply of authentic Asoka, there is substitution or adulteration with other plants. Difference in the price of the drug also created a confusion regarding the genuinity of drug. Enough availability of the drug in the market irrespective of its habitat threat is the motivation behind conducting this study.

Objective of the study

- To assess the genuinity of different market samples of Asoka (*Saraca indica* Linn) bark in Kerala, using different parametric standards. To study the qualitative and quantitative difference between Asoka bark and its samples available in crude drug market of Kerala by doing the Preliminary physicochemical analysis, HPTLC, ICPMS and Fluorescence Spectroscopy of Asoka bark available in the raw drugs markets of Kerala.

Materials and Methods

Sample collection

In the first phase of study the bark of genuine Asoka as well as the market samples were collected.

Collection of Genuine Sample

The genuine sample of Asoka was collected from its natural habitat, the tree with 42 years of age from Tripunithura and authenticated by Dr. Sandhya P, HOD of Botany department NSS College Pandalam.

Preparation of Genuine Sample

Collected and authenticated Asoka bark has been broken into small pieces and washed vigorously to get rid of dust and other external impurities. It was wiped out with a well washed clean cloth. The bark was dried in open air under shadow for seven consecutive days by spreading it in a tray. Dried bark pieces were kept in air tight container.

Coding of the drug

The dried bark pieces were powdered, stored in air tight container and labeled as Sample G (Genuine)

Asoka in its natural habitat

Asoka bark

Images of drying the sample G

On first Day

On 7th day

Collection of Market Samples

For the collection of Market, samples four different zones from our state, as north, south, central north, and central. The four market samples of Asoka *(Saraca indica* Linn) stem bark were collected from different leading suppliers of crude drug market from these zones. The four samples were cleaned, removed dust and unwanted materials from it and all are kept safely in Ziplock polythene bags.

Images of all market samples

Sample A

Sample B

Sample C

Sample D

All the 5 samples were subjected to various analytical parameters as follows:

- Foreign matter
- Determination of Moisture content
- Determination of Ash value
- Acid insoluble Ash
- Alcohol and Water-soluble Extractive
- Determination of pH
- Quantification of Tannin
- Successive Solvent Extraction
- Determination of Qualitative tests
- HPTLC
- Fluorescence Analysis
- ICPMS

The results of each evaluation are listed here:

Physical analysis of Asoka bark with API, genuine, and market samples

Showing the analytical comparison of Asoka bark with API, genuine, and market samples.

Experiment	API [1]	Sample A	Sample B	Sample C	Sample D	Sample G
Foreign matter %	Not more than 2%	3 %	4 %	4 %	2.5 %	1 %
Moisture content %	-	10 %	13 %	8 %	14 %	4 %
Total ash %	Not more than 11%	4.5 %	7 %	4.5 %	6 %	6.5 %
Acid insoluble ash %	Not more than 1%	0.5 %	0	1 %	0	0.5 %
Cold water soluble extractive %	Not less than 11%	8.8 %	12 %	8.8 %	8 %	13.8%
Alcohol soluble extractive %	Not less than 15%	3.2 %	4.8 %	5.6 %	8.8 %	16 %
Hot Water-Soluble Extractive %	-	14 %	15 %	9 %	1 %	17 %

Quantitative estimation of the pH of all samples was done with pH meter.

Enlisting the values pH

Si No	Samples	Ph
1	Sample A	6.45
2	Sample B	6.58
3	Sample C	6.67
4	Sample D	6.73
5	Sample G	6.35

Quantification of Tannin

Comparative analysis of tannin quantification of all samples with genuine and standard.

Si. No	Samples	Percentage of tannin
1	Sample A	0.41 %
2	Sample B	1.24 %
3	Sample C	1.6 %
4	Sample D	0.41 %
5	Sample G	3.83 %

Successive Solvent Extraction

Successive Solvent Extraction was done in a Soxhlet apparatus in which continuous extraction was done using four solvents choose according to their polarity. The solvents used are petroleum ether, chloroform, isopropyl alcohol and water successively. Each extract was collected by evaporating the solvents and weighed for its contents. SSE was done to genuine and all market samples. Each extract was collected by evaporating the solvents and weighed for its contents.

Comparative analysis of successive solvent extraction of all samples with genuine.

Showing successive solvent extractive values of all samples with genuine.

Si No:	Samples	Petroleum Ether	Chloroform	Isopropyl alcohol	Water
1	Sample G	1 %	0.8 %	21.4 %	3 %
2	Sample A	0.6 %	0 %	4.1 %	6.3 %
3	Sample B	0.9 %	0.4 %	5.9 %	2.1 %
4	Sample C	1.4 %	1.1 %	11.2%	7.9 %
5	Sample D	3 %	3.9 %	6.7 %	5.8 %

Qualitative analysis of all samples were done with the extracts got from petroleum ether, chloroform, isopropyl alcohol and distilled water. Tannins are strongly positive in all samples but in different extracts. Alkaloids, saponins, proteins, flavonoids, carbohydrates and phenolic compounds are present in one or in other extracts. There was no similarity between the qualitative analysis of Sample G and the remaining market samples. Comparative study of qualitative analysis of successive solvent extractive values of Asoka (*Saraca indica* Linn) with genuine and market samples are shown below:

Comparative study of qualitative analysis of successive solvent extractive values of Asoka with genuine and market samples.

Qualitative tests	Sample G	Sample A	Sample B	Sample C	Sample D
Detection of Tannin	Present in chloroform and isopropyl alcohol	Present in water.	Present in petroleum ether and water.	Present in petroleum ether and water.	Present in petroleum ether and isopropyl alcohol.
Detection of Alkaloids: • MeyersTest • Dragendroff's Test	Absent in all samples.		Present in petroleum ether.	Present in petroleum ether.	Present in water. Present in petroleum ether.
Detection of Phenolic Compounds • Lead acetate test • Ferric Chloride test	Present in chloroform	Present in water. Present in	Present in chloroform	Present in chloroform	Present in isopropyl alcohol.

		isopropyl alcohol.			
Detection of Steroid	Present in all extracts	Present in petroleum ether and water.	Present in petroleum ether and isopropyl alcohol	Present in chloroform	
Detection of carbohydrate Fehling'sTest Benedict'sTest	- Positive in isopropyl alcohol and water	-	-	- Positive in isopropyl alcohol	- Positive in isopropyl alcohol and water
Detection of Saponins	Absent in all	Present in petroleum ether.	Present in chloroform		Present in chloroform
Detection of Proteins	Present in isopropyl alcohol	Present in petroleum ether and isopropyl alcohol.	Present in petroleum ether	Present in petroleum ether and chloroform.	Present in petroleum ether.
Detection of Flavonoid Shinoda test	Present in isopropyl alcohol			Present in petroleum ether.	Present in isopropyl alcohol

Results of Heavy metal Analysis of Genuine sample of Asoka (*Saraca Indica* Linn) and market samples.

The limits of heavy metals have been explained by WHO [5].

Screening of Heavy metal content was done for the genuine sample of Asoka (*Saraca indica* Linn) and for all the market samples. First, compare the standard with genuine sample. The result obtained was enlisted below:

Analytical comparison of heavy metal analysis with standard, genuine and all market samples.

Si No:	Heavy metals (ppm)	Limits (WHO)	Sample A	Sample B	Sample C	Sample D	Sample G
1	Arsenic	3	0.12	0.06	0.05	0.07	0.06
2	Cadmium	0.3	0.06	**1.13**	BDL	BDL	BDL
3	Lead	10	1.81	BDL	1.07	0.06	0.34
4	Mercury	1	BDL	BDL	1.62	BDL	BDL

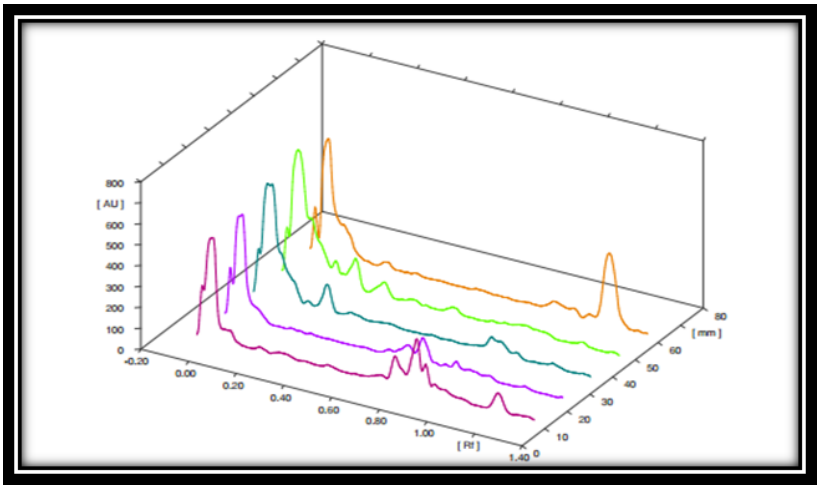
HPTLC

(High-Performance Thin Layer Chromatography)

HPTLC analysis of genuine heart wood of Asoka (*Saraca Indica* Linn) and the market samples was done.

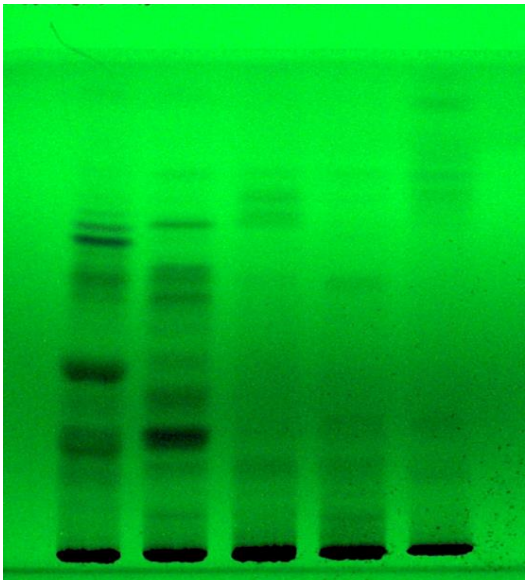
1 g powder of stem bark of Asoka (*Saraca indica* Linn) was weighed, extracted with 10 ml methanol and spotted as 15 micro-liter. The stationary phase was Merk, 1.05554.0007, TLC Silica gel 60 F 254, 8 x10 cm aluminium sheet. The mobile phase was toluene: ethyl acetate: formic acid: methanol (3:3:0.8:0.2). The development of the plate is done in the CAMAG 10 x 10 cm twin trough chamber and visualized under UV at 254 nm and 366 nm after derivatization using iodine vapour. The number of peaks as well as the Rf values were noted. The observations were as follows.

Graph Presenting the peaks of all samples



Results of HPTLC analysis are given below:

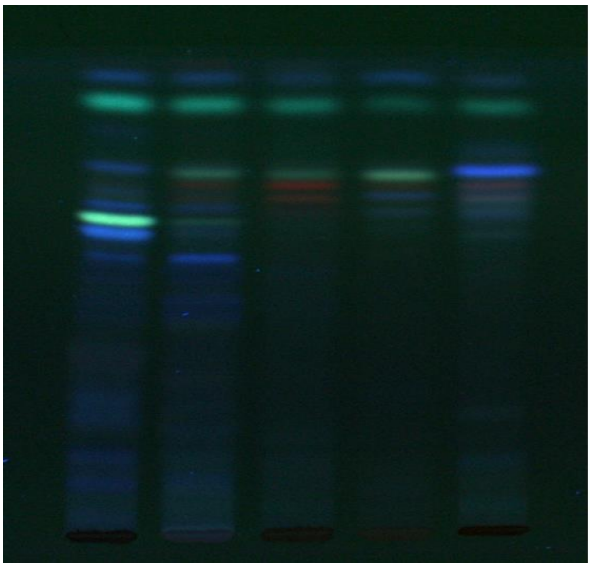
Visualized under UV at 254 nm



Sample G, A, B, C and D successively.

The development of the plate was done in the CAMAG 20 X 10 cm chamber and visualized under UV at 254 nm in mobile phase toluene: ethyl acetate: formic acid: methanol (3: 3: 0.8: 0.2) on 60 ^{0}C for 5 minutes.

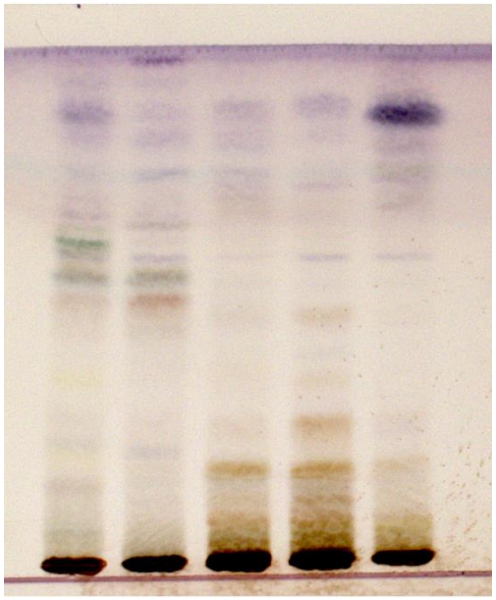
Post- Chromatographic Derivatization at 366nm



Sample G, A, B, C and D successively.

Derivatization was done with anisaldehyde-sulphuric acid reagent on temperature 120 ^{0}C for 20 minutes.

Derivatization at white light at 366 nm.



Sample G, A, B, C and D successively.

Image of 5 samples at 366 nm after derivatization using anisaldehyde-sulphuric acid reagent with white visible light.

Total no. of peaks and areas covered in each sample at 254 nm are tabulated below

Samples	No. of Peaks	Area (AU)
Sample G	8	37,781.4
Sample A	14	35,908.3
Sample B	10	29,320
Sample C	11	36,674.3
Sample D	12	47,630.5

HPTLC analysis of powder of stem bark of Asoka (*Saraca indica* Linn) in methanolic extract reveals 8 peaks with total area of 37,781.4 A.U at 254 nm. Among them the two major peaks were seen at Rf -0.01 with an area 18607.9 A.U (49.25 %) and Rf 1.17 with an area 12630.9 A.U (33.43 %). Sample A with major peaks were seen at Rf -0.02 with an area

14411.3 A.U (40.13 %) and Rf 0.84 with an area 6360.7 A.U (17.71 %). Sample B with major peaks were seen at Rf-0.01 with an area 16743.7 A.U (57.11 %) and Rf -0.06with an area 2599.9 A.U (8.87 %). Sample C with major peaks were seen at Rf -0.00 with an area 16148.0 A.U (44.03 %) and Rf -0.02 with an area 10984.9 A.U (29.95 %). Sample D with major peaks were seen at Rf -0.01 with an area 22527.0 A.U (47.30 %) and Rf 0.04 with an area 9801.3 A.U (20.58 %). The areas covered by the market samples were 35,908.3 A.U, 29,320 A.U, 36,674.3 A.U, 47,630. 5 A.U respectively.

Fluorescence analysis

Fluorescence analysis of genuine Asoka and all the market samples were done and shown below:

Comparative fluorescence analysis of market sample with genuine in visible light.

1	Powder as such	Brown	Light brown	Light brown	Brown	Light brown
2	Powder + 1 N NaOH in water	Blackish brown	Blackish brown	Dark brown	Blackish brown	Reddish brown
3	Powder + 1 NaOH in methanol	Blackish brown	Dark brown	Brown	Brown	Brown
4	Powder + 50% KOH	Black	Brown	Brown	Dark brown	Light brown
5	Powder + 1 N HCL	Brown	Brown	Brown	Reddish brown	Light brown
6	Powder + 50% H_2SO_4	Blackish brown	Blackish brown	Blackish brown	Blackish brown	Brown
7	Powder + 50% HNO_3	Reddish brown	Reddish brown	Reddish brown	Reddish brown	Reddish brown
8	Powder + Con: HNO_3	Reddish brown	Reddish brown	Reddish brown	Reddish brown	Reddish brown
9	Powder + Con: H_2SO_4	Black	Black	Black	Black	Black
10	Powder + Iodine water	Black	Black	Brown	Dark brown	Brown

Comparative fluorescence analysis of market sample with genuine in short wave.

1	Powder as such	Greenish brown	Greenish brown	Greenish brown	Greenish brown	Greenish brown
2	Powder + 1 N NaOH in water	Black	Black	Black	Black	Black
3	Powder + 1 NaOH in methanol	Black	Black	Black	Black	Black
4	Powder + 50% KOH	Black	Black	Black	Black	Black
5	Powder + 1 N HCL	Black	Black	Black	Green	Green
6	Powder + 50% H_2SO_4	Dark green	Dark green	Dark green	Black	Dark green
7	Powder + 50% HNO_3	Black	Black	Black	Black	Black
8	Powder + Con: HNO_3	Black	Black	Black	Black	Black
9	Powder + Con: H_2SO_4	Black	Black	Black	Black	Black
10	Powder + Iodine water	Black	Black	Green	Black	Green

Comparative fluorescence analysis of market samples with genuine in long wave.

1	Powder as such	Brown	Brown	Brown	Brown	Brown
2	Powder + 1 N NaOH in water	Black	Black	Black	Black	Black
3	Powder + 1 NaOH in methanol	Black	Black	Black	Black	Black
4	Powder + 50% KOH	Black	Black	Black	Black	Black
5	Powder + 1 N HCL	Black	Black	Black	Black	Black
6	Powder + 50% H_2SO_4	Black	Black	Black	Black	Black
7	Powder + 50% HNO_3	Black	Black	Black	Black	Black
8	Powder + Con: HNO_3	Black	Black	Black	Black	Black
9	Powder + Con: H_2SO_4	Black	Black	Black	Black	Black
10	Powder + Iodine water	Black	Black	Black	Black	Black

Discussion

a) Foreign matter

Foreign matter present in genuine and market samples was evaluated. The percentage of foreign matter was higher in market samples when compared with the genuine sample. It shows that there will be foreign organs or foreign elements in market samples. Visible inspection is one of the common methods for detecting defects or foreign matter. There were moulds and animal contamination in all market samples and more in Sample B. The foreign matter decreases the quality and efficacy of formulations.

b) Determination of Moisture content

Moisture content determination is one of the important methods, in plant drug standardization. Excess moisture in a sample, can result in the breakdown of the important constituents by enzyme activity and may also encourage growth of yeast and fungi during storage. The result showed Sample G had 4 % w/w while marketed samples showed much higher percentage of moisture content as 10 %, 13 %, 8 % and 14 %. Low moisture content is always desirable for the higher stability of drugs. The moisture content estimation revealed that Sample G has less amount of water content. That shows that the traders might have not allowed the collected material to dry properly before marketing. High moisture content will deteriorate the formulations.

c) Determination of Ash value

Ash value represents the amount of inorganic salts occurring in the drug or adhering to the drug or deliberately added to the drug. The total ash of Sample G was 3.5% w/w whereas Samples A, B, C, D showed 4.5%, 7%, 4.5% and 6% respectively. It indicates that Samples B and D contain more amount of inorganic matter such as Na^{+}, K^{+} and Ca^{++} .

d) Acid insoluble Ash

Acid insoluble Ash value indicates silica impurities or earthly material contamination of crude drugs. A higher limit of acid insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high. The acid insoluble ash of Sample G was 0 and the same value has been got on to Samples B and D whereas Sample A and Sample C shown 0.5% and 1% w/w respectively. Thus, can be concluded that genuine and all market samples had no or very less silica impurities.

e) **Alcohol Soluble Extractive**

Alcohol Soluble Extractive values of Sample G were 16% and others as 3.2%, 4.8%, 5.6% and 8.8%. Here only genuine samples satisfy the API value as not less than 15% alcohol soluble extractive is present in Asoka stem bark powder. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating.

f) **Water Soluble Extractive**

In Ayurvedic formulations water extracts of various crude drugs are used for therapeutic purpose. Water is universal solvent. Extractive values were determined with different solvents to get the better solubility of drug. The water soluble extractive value of Sample G was 13.8 % and Sample B also shown a near value as 12 % where as other samples shown 8.8 % (Samples A & C), and Sample D shown 8 %. These values indicates that the samples A, C and D have some similarity or may be one.

g) **Hot Water Soluble Extractive**

The values for hot water soluble extractives are higher than those of cold water. The difference in solubility is due to hydrolysis and corresponding increase in solubility of substance during boiling. The corresponding values are 17 %, 14 %, 15 %, 9 % and 1 % for Sample G, A, B, C and D. Less extractive value indicates addition of any exhausted material or adulteration.

h) **Determination of pH**

Quantitative and qualitative estimation of pH was done. Sample G, Sample A, Sample B, Sample C and Sample D had pH of 6.35, 6.45, 6.58, 6.67, and 6.73 respectively.

i) **Quantification of Tannin**

Tannins are one of the most widely occurring groups of secondary metabolites present in different families of higher plants. Tannins are of two types, they are hydrolysable tannin and condensed tannin. Here Asoka contains condensed tannin. They are also known as non-hydrolysable tannins, phlobatannins or proanthocyanidins. Tannin was quantified with indigo carmine method. Result obtained are as follows; 0.41, 1.24, 1.6, 0.41 and 3.83 to Sample A, B, C, D and G respectively.

j) **Successive Solvent Extraction**

Successive solvent extraction is a technique by which the drug is successively extracted with various solvents as per their polarity from a non-polar solvent to a more polar solvent. The various extractives obtained from the crude drug are indicative of their approximate measures of chemical constituents. The extractive values of powder of Sample G using solvents petroleum ether, chloroform, isopropyl alcohol and distilled water were 1 %, 0.8 %, 21.4 % and 3 % respectively. From this values, it was clear that most of the active principles of bark had been extracted out through isopropyl alcohol, due to its .That we know that most of the Asoka preparations had been in Asava and Arishta form. Sample A shown 0.6 %, 0 %, 4.1 % and 6.3 % successively. Sample B shown 0.9 %, 0.4 %, 5.9 % and 2.1 %. Sample C had extractive values 1.4 %, 1.1 %, 11.2 % and 7.9 %. Sample D shown 3 %, 3.9 %, 6.7 % and 5.8 % respectively.

k) **Determination of Qualitative tests**

A qualitative analysis of ethanolic extracts of genuine and market samples were done. This screening was very relevant since the pharmacological activity of a drug depends mainly on its chemical constituents. This screening method showed the presence of tannin, phenol, carbohydrate, saponin, alkaloids, proteins, flavonoids and steroids in Sample G. Some of these constituents were present in the market samples but the concentration was lower than that in the genuine sample. Tannins was present in all samples but in different extracts, most of the samples shown positive in petroleum ether and water. Alkaloids were absent in genuine but present in Sample B, C and D. Phenolic compounds shown presence in chloroform extract in genuine and Sample C and all other samples in isopropyl and water. Carbohydrates was present in isopropyl and water in all samples. Steroids and Proteins were present in all samples. Flavonoid presence was seen in isopropyl to genuine and Sample D where as in petroleum ether for Sample C.

l) **HPTLC**

HPTLC chromatograms of genuine sample and market samples were recorded and compared with the genuine. The fingerprint data of the Sample G can be certainly used as standard to compare the quality of the market samples. All other samples are compared with the genuine. HPTLC fingerprinting analysis shows Sample G have 8 peaks with an area of 37,781.4 A U and Rf value more than the marketed samples. Sample A, Sample B, Sample C, Sample D

had shown 14, 10, 11 and 12 number of peaks respectively. Sample G had 2 major peaks with major peaks were seen at Rf-0.01 with an area 18607.9 A.U (49.25 %) and Rf 1.17 with an area 12630.9 A.U (33.43%). Sample A with major peaks were seen at Rf-0.02 with an area 14411.3 A.U (40.13 %) and Rf 0.84 with an area 6360.7 A.U (17.71 %). Sample B with major peaks were seen at Rf-0.01 with an area 16743.7 A.U (57.11 %) and Rf -0.06 with an area 2599.9 A.U (8.87 %). Sample C with major peaks were seen at Rf-0.00 with an area 16148.0 A.U (44.03 %) and Rf -0.02 with an area 10984.9 A.U (29.95 %). Sample D with major peaks were seen at Rf -0.01 with an area 22527.0 A.U (47.30 %) and Rf 0.04 with an area 9801.3 A.U (20.58 %). The areas covered by the market samples were 35,908.3 A.U, 29,320 A.U, 36,674.3 A.U, 47,630. 5 A.U respectively.

m) **Fluorescence analysis**

Fluorescence analysis is an important standardization parameter. Many phytochemicals present in plant materials shown fluorescence when examined under UV light. For each compound colour of fluorescence is specific. The distinctive color changes in treated powder observed under UV light examination and the results. The light reflected by the market samples was different from the genuine.

n) **ICPMS**

The genuine as well as the market samples were screened for the presence of heavy metals such as arsenic, cadmium, lead and mercury. Inductively coupled plasma mass spectroscopy was done. The values were recorded in ppm. It was found that Sample B contained 1.13 ppm of cadmium. All other samples and genuine showed the presence of heavy metals as in the permissible limit only.

Thus, it was evident from the study that market samples were of inferior quality and were adulterated with the stem bark of some other botanical sources. Such adulterated and substandard commercial samples of Asoka (*Saraca Indica* Linn) cannot bring the desired pharmacological effects.

Summary

The Physicochemical parameters such as Foreign matter, Moisture content, Total ash, Acid insoluble ash, Cold water soluble extractive, hot water soluble extractive and Alcohol soluble

extractive values were evaluated. The results obtained were, only genuine sample satisfied all the standards mentioned by API.

Foreign particle material identification is a key step in the drug standardization and quality control of drugs and is used to identify contaminants in drugs which might affect the quality and safety of a drug product. The amount of foreign matter should not be higher than the standard regulated for each drug according to the pharmacopoeia monograph. If it exceeds the limits, quality of drug deteriorates. The percentage of foreign matter was higher in market samples when compared with the genuine sample.

Total ash and acid insoluble ash values doesn't show any much verifications as it shows that presence of inorganic contents and silica impurities or earthly materials were very less in all samples. The extractive values of the crude drug determine the quality as well as the purity of the drug. Here the extractive values were found to be very less as compared to the genuine sample. Less extractive value indicates addition of any exhausted material or adulteration.

Estimation of pH was done both quantitatively and qualitatively. On Quantitative estimation colour noted on litmus paper was. Qualitatively genuine samples shown light acidic in nature and all other market samples showed values other than genuine.

Quantification of tannin was done using indigo carmine method. Tannins are complex secondary metabolites having various medicinal properties but difficult to isolate in pure form. Condensed type of tannin is present in Asoka (*Saraca indica* Linn) and that in 0.57 % to 7.85 %. Result obtained are as follows; 0.41, 1.24, 1.6, 0.41 and 3.83 to Sample A, B, C, D and G respectively.

As the next innovative method, we can make use of chemical constituents in the drug as specific markers to ensure its authenticity. Analysis of marker compounds requires extensive scientific procedure and SSE (Successive Solvent Extraction) is one among them. Successive Solvent Extraction is a commonly employed technique for the extraction of active substances from crude drug, involving the use of different solvents. Extraction is done with solvents petroleum ether, chloroform, isopropyl alcohol and distilled water. It is an innovative technique to distinguish the adulteration of drug samples. Extractive values also show difference in solubility of samples in different solvents. The extractive values of all the market samples were completely different from the genuine.

The extractives obtained by successive solvent extraction were analyzed qualitatively for identification of various plant constituents. The qualitative analysis also showed significant difference in tannin, phenol, carbohydrates, saponins, alkaloids, protein, flavonoids and steroids. Some of these constituents were present in the market samples but the concentration was lower than that in the genuine sample.

HPTLC (High performance thin layer chromatography) analysis of powder of stem bark of Asoka (*Saraca indica* Linn) and the market samples in methanolic extracts were done. Sample G revealed 8 peaks. Among them two major peaks were seen at Rf 0.01 and -0.06 as 18607.9 A U and 2414.3 A U area respectively at 254 nm. All other market samples shown 14, 10, 11 and 12 number of peaks respectively. The total area of Sample G was 37,781.4 A U whereas market samples had 35,908 A U, 29,320 A U, 36,574.3 A U, and 47,630. 5 A U respectively. All market samples were had different number of peaks with a total area which is extremely different from the genuine.

Fluorescence analysis was done to the genuine Asoka (*Saraca indica* Linn) and all other market samples. Fluorescence studies helps in the identification of a drugs that are more or less difficult to distinguish. All the market samples shown different colours from the genuine.

The genuine as well as the market samples were screened for the presence of heavy metals such as arsenic, cadmium, lead and mercury. It was found that Sample B contained 1.13 ppm of cadmium. All other samples and genuine showed the presence of heavy metals as in the permissible limit only.

CONCLUSION

The current study was done to assess the genuinity of Asoka bark available in the raw drug markets of Kerala in the name of Asoka. This study mainly aimed in the qualitative and quantitative difference between the genuine Asoka and the market samples and to find out the similarities and differences between authentic and market samples.

Asoka bark and its different formulations are very much popular and have tremendous action in the treatment of gynaecological disorders. Manufacturers across the country use about 2,250 million tons of bark and flowers annually, and its need is increasing at the rate of 15% annually. In India only few farmers are cultivating Asoka and they can never supply this much tons of Asoka. Based on the conservation status, Asoka has been included in the vulnerable category by IUCN and one among the 32 priority species listed by the National

Medicinal Plant Board. That means it was declining from its natural habitat too. But when coming to the raw drug markets, there is no scarcity of the same and it was easily available. Hence the genuineness of the samples are doubted and this is the motivation behind the study. The selection of the topic seems relevant.

All the market samples and the genuine were subjected to all the Phyto-chemical evaluations. Various parameters like Foreign matter, Total ash, Acid insoluble ash, Quantification of tannin and pH, High-performance thin layer chromatography, Inductively coupled plasma mass spectrometry and Fluorescence analysis were done. Out of them, sample G was passed in all parameters and market samples were failed in one or more than one parameter for the standardization of raw drugs mentioned in API. Standardization of herbal drugs is essential in order to assess the quality of drugs, based on the concentration of their active principles.

This study of Phytochemical Evaluation of different Market samples of Asoka (*Saraca Indica* Linn) stem bark in Kerala can be concluded as:

- The Physical and Phytochemical profile of market samples of stem bark of Asoka showed remarkable variations from the genuine drug Asoka (*Saraca indica* Linn), and it's a clear indication of Adulteration or Substitution with the Asoka stem bark.
- The stem bark of Asoka may be adulterated with some other botanical sources.
- Samples A, C and D is not Asoka whereas Sample B had shown some similarity with the genuine.
- Such adulterated and inferior quality drugs may decrease the efficacy of formulations.
- Moreover, such adulterated and substandard drugs available in the commercial raw drug markets cannot bring the top-rated pharmacological utility and sometimes may lead to severe side effects.

This study inferred that there is mass decline of Asoka from the wild and the Asoka bark what we getting from the raw drug markets are adulterated or substituted with some other botanical sources which are Phytochemically very similar to the same. Hence it is very essential to cultivate more Asoka and protect the existing population. For that one can make use of the both *in situ* and *ex situ* conservation. Mass propagation of Asoka tree can ensure regular and genuine supply of drug to the Ayurvedic physician and manufacturers. This will also reduce the chances of adulteration.

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