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Study of *Ashoka (Saraca Asoca)* Bark w.s.r. to its samples available in the crude drug market of Bombay

Anil Kumar Shukla

Professor & Head, Dept of Dravyaguna, Mansarovar Ayurved Medical College, Hospital & Research Center, Bhopal, Madhyapradesh, India.

ABSTRACT

Ashoka is one of the most legendary and sacred trees of India. The *Ashoka* bark has been very widely used in Indian System of Medicine. It has very good effects in Uterine, genital and reproductive disorders. *Ashoka* bark is one of the top 20 herbs traded in Ayurveda and Herbal Industry being main ingredient in almost all formulations to treat Gynaecological disorders. Due to more demand and less supply of authentic *Ashoka*, there is substitution or adulteration with other plants. In this study of *Ashoka* bark with special reference to its samples available in the crude drug market of Mumbai, we determined method and time of collection of the authentic *Ashoka* bark, its organoleptic characteristics, Pharmacognostical and physic-chemical along with market samples. A great difference was observed in authentic *Ashoka* bark and marketed samples. Reference *Ashoka* bark collected from Sanjay Gandhi National Botanical Garden, Mumbai as per procedures described in Charak Samhita Vimansthana chaper 8. Other five samples of *Ashoka* bark available in name of *Ashoka* were collected from different locations of Mumbai crude drug Market. All six samples were properly powdered, sieved and kept in air tight containers for further study. In this study, emphasis is led upon standardisation of *Ashoka* - *Saraca asoca* (Roxb.) and its samples available in the crude drug market.

Key words: *Ashoka, Saraca asoca, Adulteration, Pharmacognostical study, Phyto-chemical study.*

INTRODUCTION

Ayurveda the Indian system of medicine dating back to the Vedic period (4500 – 1600 B.C.) has always been an integral part of Indian culture. The treaties believes that it is not just science of treatment of the disease but in composes the entire gamete of happy human life and include in this are the physical metaphysical and spiritual aspect of human life.

Address for correspondence:
Dr. Anil Kumar Shukla
Professor & Head, Dept of Dravyaguna, Mansarovar Ayurved Medical College, Hospital & Research Center, Bhopal, Madhyapradesh, India.
E-mail: dranilshukla@gmail.com

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Ayurveda the traditional science of heath based on universal truth and natural laws. Man and environment believed to be integral part of each other and both being composed of five basic elements (*Panchamahabhuta*) which can be perceived by the 5 sense organs.[1,2]

Physician, drug, nurse and patients are collectively called as '*Chikista Chatuspada*' (four quadrant of treatment) drug being the integral part and prime tool of *Chikista*, its purity genunity and knowledge towards its therapeutic property are very much necessary. In Ayurveda the source of drugs are from plant Kingdom, animal kingdom and minerals of all these drugs originated from plant Kingdom consisting the major part of the Ayurvedic drugs. Due to rapid untoward reactions and side effects with synthetic western medicine, more and more people are now realizing that cure from natural products are better one. In Vedic period there was no controversies in identification of drug because in ancient time the system of a study was by *Gurukul Parampara*, which

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was combination of both system of learning that formal and informal learning by which the students and teachers had very good report among themselves and they were in *Ashramas*, they could find many drug identified them in the nearby forest, as start by teachers. As time passed system of education change due to discontinuation in informal learning about identification of medicinal plant has resulted in controversies.[4]

The process of admixuring the non-drug or other part of an official drug is called adulteration. An adulterant is substance which may be an organic or inorganic matter used for these process. The inorganic includes stone, dust, mud etc. The organic adulterant include other part of the official drug are entirely different drug etc. The art of adulteration become specific that even the crude drug sellers prepares spurious drugs and admixture it with genuine one, often even the learned *Vaidyas* find it difficult to differentiate them.

Hence it is essential to evolve and develop new scientific technique are tested to identified the adulterants. The drug which are non-standardised may not be of value for the purpose for which they are being used. Thus quality of the drugs would not hold true for the drug which are not standardised as it has been mentioned in *Charak Samhita Sutrasthan*.

About the qualities of single drugs as - Abundance (availability in large amount), suitability, can be used in multiple form and potency are the four qualities of drug. The word standardisation includes various field of study from origin of plant to clinical application. It is a vast and very important study that involves various scientific disciplines.

In the present era standardisation has become prime importance in view of doubtless expectances of Ayurvedic drug. While establishing quality control are standardisation method of raw materials be should give thought be the aspect selection of drug. There are different ways to assess the quality of raw material as well as finished product by various organoleptic, microscopic, physico-chemical analysis and clinical efficacy of the drug.[5]

Figure 1: *Ashoka* (*Saraca Asoca* Roxb.) tree with flowers

According of Ayurvedic literature it is has found that *Ashoka* bark has very good effect in treating gynaecological disorders.

The word *Ashoka* means "without sorrow", a reference to reputation of its bark for keeping a woman healthy and youthful (Figure 1). Formulation *Ashokarishta* is well known drug, which is one of the top twenty selling Ayurvedic medicines. Mumbai, the economical capital of India is the largest market of Ayurvedic crude drug and finished products. After visiting almost all the leading pharmacist and Ayurvedic institution all over the India during present research, Ashoka tree was not noticed in garden adjacent to any pharmacy or in any botanical garden. In market survey of raw drugs supplies in Mumbai there are variations in *Ashoka* bark size of thickness, colour, odour and price.

It is clearly visible to the unaided that all the samples of *Ashoka* bark powder various sources have different colours than the authentic one. Hence genuineness of the samples is doubted. The *Ashoka* bark is used in nearly 50 ethical and more than 150 proprietary

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formulations for many disorders. Its habitat is West Bengal, South India, and Uttar Pradesh only. Therefore, its non-freely availability and effectiveness in many diseases have made it suitable drug for adulteration and hence its standardisation becomes mandatory.

The present topic is study of *Ashoka* bark (*Saraca asoca* roxb) with special reference its samples available in the crude drug market in Mumbai has been selected by the above factors. In Ayurveda, there are various methods to assist the quality of raw materials basically most of them are subjective and required definite objective criteria to enhance knowledge about them.

The objective criteria require extreme expertise and repeated long term experimentations which cannot be carried out in limited period. In this vast field of research work, we intended to study only the qualitative and quantitative difference between the reference *Ashoka* bark and various samples sold under the name *Ashoka* bark in Mumbai, by Pharmacognostical, Physico- chemical, HPTLC and ICAPES.[6-10]

Objective of the Study

To study the qualitative and quantitative differentiate between authentic Ashoka bark and its samples available in crude drug market of Mumbai and to find out the similarities and differences between authentic and market samples.

Material and Methods

Collection, identification and preparation of reference crude drug was done as per Ayurvedic principles. Five different samples were procured from the markets of different locations of Mumbai. Organoleptic evaluations were done by Ayurvedic parameters. Macroscopic, microscopic study as well as physico-chemical and phyto-chemical evaluation of whole *Ashoka* bark and its powder were done. Fluorescence study under UV light, high performance thin layer chromatography (HPTLC) and inductively coupled plasma atomic emission spectroscopy (ICAPES) were performed for analysis.

Collection and identification and preparation of reference crude drug and other samples[11,12]

Collection

Reference sample of *Ashoka* - *Saraca asoca* Roxb. Bark was collected after authentication from Sanjay Gandhi National Botanical Garden under supervision of Conservator of Forest Mr. Nigam. Prior to collection day "Awahana Mantra" was done as mentioned in Ayurvedic text Charak Samhita. Next day early morning in *Pushya Nakshtra* of *Chaitra Masa* bark was collected.

Identification

Further identification was done from reference Herbarium library, ST. BLATTER herbarium by Dr.(Mrs) Almeida, Professor & Head, Department of Botany from, Department of Botany, St. Xaviers College, Mumbai.

Preparation of Reference Sample (Drug)

Collected and authenticated *Ashoka* Bark was broken in small pieces and washed vigorously to get rid of dust and other external impurities. It was wiped out with well washed clean linen cloth. The bark was dried in open air under shadow for seven consecutive days by spreading in thin layer in a tray. Dried Bark pieces were kept in air tight container for further studies.

5 other samples sold as *Ashoka* bark powder were collected from different leading suppliers from crude drug market of Mumbai.

The all 6 samples were powdered and were passed through Sieve size no. 60 (ISS 250, BSS 60) and stored in air tight container.

Coding of the drugs

Samples of drugs were labelled as follows: Reference Sample as R.S. and 5 Market samples as MS1, MS2, MS3, MS4, and MS5 respectively.

All the 6 samples were subjected to various analytical parameters as follows.

- Organoleptic evaluations (Macroscopic Examinations)[13,14]
- Microscopic examinations

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- Physico-chemical methods: Loss on drying at 110°C, pH value, ash value, acid insoluble ash, water insoluble ash, water soluble extractive, alcohol soluble extractive, chloroform extractive value.
- Qualitative tests for various Phytoconstituents like presence of alkaloids, glycosides, sugars, resins, saponins, tannins, phenols, steroids, starch, calcium and iron were performed.
- Fluorescence Characteristics of Powdered Bark under U-V Light.[14]
- Inductivity Coupled Plasma Source for Atomic Emission Spectrophotometry (I.C.A.P.E.S.).[13]
- High Performance Thin Layer Chromatography (H.P.T.L.C.)[10]

RESULTS AND DISCUSSION

Sparsha pariksha for all six samples showed *Ruksha Sparsha*. The *Rasa* of reference sample was *Kashaya* and *Tikta* where as MS1, MS4, MS5 were *Tikta Rastamaka*. In all the six samples no specific odour was noted (Table 1 & 2)

When powders were treated with water at room temperature reference sample showed, yellowish brown colour whereas marketed sample gave brown colour. At boiling temperature RS gave browncolour while others deep brown. (Table 3)

When powders were treated with Ghrita at room temperature and at boiling temperature, R.S. showed yellowish red and deep brown colour respectively. The marketed samples showed brown colour at room temperature and deep brown at boiling temperature. (Table 4)

Table 1: Colour of the powdered drugs

Samples	Colour of the Powder
RS	Brown
MS1	Light Brown
MS2	Brown
MS3	Light Brown
MS4	Deep Brown
MS5	Blackish Brown

Table 2: Taste of the Powdered Drugs

Samples	Taste of the Powder
RS	*Kashya (++), Tikta (+)*
MS1	*Tikta (++), Kashaya (+)*
MS2	*Tikta (++), Kashaya (+)*
MS3	*Tikta (+), Kashaya (++)*
MS4	*Tikta (+), Kashaya (+)*
MS5	*Tikta (+), Kashaya (++)*

Table 3: Behaviour of the samples with water

Samples	At room temperature	At boiling temperature
RS	Yellowish Brown	Brown
MS1	Brown	Deep Brown
MS2	Brown	Deep Brown
MS3	Brown	Deep Brown
MS4	Brown	Deep Brown
MS5	Brown	Deep Brown

Table 4: Behaviour of the powdered drug with *Ghrita.*

Samples	At Room Temperature	At Boiling Temperature
RS	Yellowish Brown	Coffee Colour
MS1	Brown	Deep Brown
MS2	Brown	Deep Brown
MS3	Brown	Coffee Colour
MS4	Brown	Deep Brown
MS5	Yellowish Brown	Coffee Colour

The morphology i.e. appearance of all marketed samples are different from reference sample, showed that the some other drug (adulterant) used in place of original *Ashoka* bark (*Saraca asoca*). Microscopic study of powdered drugs showed some of the characters which are found in RS they are absent in marketed samples. Organoleptic evaluation showed all samples are different from RS in colour, taste, and

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reaction with water and fire. The moisture content of RS was less than MS, showed MS had more amounts "Jala Mahabhoot". Thus we can conclude that the traders might not have allowed the collected material to dry property before marketing. Total ash of RS and MS3 were more than other MS. It indicates RS and MS3 contain more amounts of inorganic matters. AIA of RS, MS2 and MS4 had approximately similar values.

Study of powder drug

Microscopic examination of powdered reference sample showed presence of plenty of stone cells calcium oxalate crystal sheath, starch grains as identifying characters.Marketed samples showed plenty of fibres with fewer amounts of other characters (Table 5).

Table 5: Characters observed during powder study

Characters	RS	MS1	MS2	MS3	MS4	MS5
Cork Cells	+	+	+	+	+	+
Calcium oxalate cells	+	+	-	-	-	-
Calcium oxalate crystal sheath	++	-	-	-	-	-
Phloem Fibres	+++	-	+	+	+	+
Stone Cells	++	+	+	+	+	+
Pith cells	+	-	-	-	-	-
Starch	+	-	-	-	-	-
Sieve tubes and companion cells	+	-	-	-	-	-
Medullary rays	+	+	+	+	+	+

Determination of moisture content of loss of drying

Moisture content determination is one of the important methods, in plant drug standardisation. 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. One of the methods of moisture determination is loss on drying. The resultshowed R.S. had 10.30% w/w while marketed samples showed much higher percent of moisture content (Table 6).

Table 6: Determination of Moisture Content

Samples	Moisture Content (%w/w)
RS	10.30
MS1	25.92
MS2	21.22
MS3	15.70
MS4	15.46
MS5	19.30

Moisture up to 12% is usually not considered as excessive. The moisture content by loss on drying method revealed that the R.S. has less amount of '*Jala Mahabhoota'* (i.e. water content) compared to the marketed samples. That shows that the traders might have not allowed the collected material to dry properly before marketing.

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 as adulteration. The total ash of RS was 6.17% w/w whereas sample MS1, MS2, MS4, MS5, showed less amount of ash, MS3 showed more ash value (13.30%). It indicate that R.S. and MS3 contain more amount of inorganic matter such as NA^{+}, K^{+} and Ca^{++} than other samples (Table 7).

Table 7: Determination of different ash values

Samples	Total Ash % w/w	Acid Insoluble Ash % w/w	Water Soluble Ash % w/w
RS	6.17	1.01	2.00
MS1	3.85	0.99	1.42
MS2	3.55	2.00	3.57
MS3	13.30	7.71	2.68
MS4	2.16	1.02	0.25
MS5	5.56	3.84	2.00

Acid insoluble ash

The results shows that AIA of reference sample was 1% w/w, MS2, MS4 had approximately same values, whereas MS3, MS4, had approximately same values,

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whereas MS5 showed different % w/w of AIA (Table 7).

Water soluble ash

Water soluble ash RS and MS3 was exactly same where as others showed different values. Ash value denotes the concentration of "Parthiv tatva" (earthy matter) (Table 7).

Extractive values

In Ayurvedic formulations water extracts of various crude drugs are mainly used for therapeutic purpose. Water is an universal solvent. Extractive values were determined with different solvents to get the better solubility of drug. Water soluble extractive values were nearly same in RS, MS2 and MS3 (Table 8).

Alcohol soluble extractive value of RS was 6.27% w/w while other showed less amount of extractive value. Petroleum either extractive values of RS was 6.45% w/w where as MS1 and MS4 have more extractive values. MS2 and MS3 have less amount extractive values. Chloroform extractive value of FRS and all MS were almost same except MS5 which had less amount of chloroform extractive.

Table 8: Determination of different extractive values

Samples	Water Extract % w/w	Ethanol Extract % w/w	Chloroform Extract % w/w	Petroleum Ether Extract % w/w
RS	5.87	5.33	6.27	6.45
MS1	27.66	65.31	4.71	12.06
MS2	6.71	60.53	5.70	5.17
MS3	4.47	59.16	4.62	3.82
MS4	9.23	27.60	5.00	12.38
MS5	13.30	42.80	2.87	9.34

pH value determination

pH of RS and MS4 are acidic (6.32 and 6.00, respectively) While MS1, MS2 MS3, MS5 had strong acidic PH. According to previous research work done in relation with Virya and pH by Prof. Dhyani acidic pH indicates "Sheeta Virya" (Table 9).

Table 9: Determination of pH value

Samples	Moisture content (%w/w)
RS	6.32
MS1	5.02
MS2	5.30
MS3	5.74
MS4	6.00
MS5	5.33

Phytochemical screening

All the six samples (RS & 5 MS) were tested for various phytoconstituents by performing qualitative chemical tests (Table 10).

Table 10: Behaviour of the powdered on treatment with some chemical reagents

Reagent	RS	MS1	MS2	MS3	MS4	MS5
Aq. Iodine	Remains almost brown as starch is present in very small	Brown	Yellow	Brown	Black	Brown
Nitric acid (50% v/v)	Remains Brown	Dark Brown	Brown	Dark Brown	Brown	Brown
Picric acid Saturated solution	Remains Brown for 10 minutes and then turns slowly orange yellow	Orange Yellow	Brown	Orange Yellow	Yellow	Brown
KOH 5% (Aq. Solution)	Deep chocolate colour	Black	Black	Brown	Chocolate colour	Brown

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Determination of alkaloids, tannins, saponins, glycosides and phenols

Alkaloids were absent in all the samples. Test for tannin was strongly positive in RS, MS1, MS2, MS3, and MS5 showed presence of tannins, where as they were as they were absent in MS4. Total tannin content (% w/w was same in RS, MS1 and MS4. While less in MS2 and MS5.Steroids were present in all the samples.Saponins were present in all the samples. Glycosideswere found only in RS and absent in all the marketed samples. Phenols were present in RS, MS1, MS2, and MS3 and absent in MS4 and MS5. All the samples showed presence of carbohydrates. Total glycoside content was 0.2% w/w in RS while they were absent in all the marketed samples. Calcium compounds were present in all the samples. Iron compounds were present in all the samples (Table 11-23).

Table 11: Determination of tannins

Samples	Colour of Precipitate	Results
RS	Dark Yellowish Brown	+++
MS1	Yellowish Brown	+
MS2	Yellowish Brown	+
MS3	Yellowish Brown	+
MS4	Brown colour	-
MS5	Yellowish Brown	+

Table 12: Identification of alkaloids

Samples	Colour	Results
RS	Brown	Absent
MS1	Red	Absent
MS2	Red	Absent
MS3	Brown	Absent
MS4	Brown	Absent
MS5	Red	Absent

Table 13: Identification of Steroids

Samples	S-R Test	L-B Test
RS	+++	+++
MS1	++	++
MS2	++++	++
MS3	++	+
MS4	++	+
MS5	+	+

Table 14: Identification of Saponins

Samples	Results
RS	+++
MS1	+
MS2	+++
MS3	+
MS4	+++
MS5	++++

Table 15: Identification of Glycosides

Samples	Colour	Results
RS	Brownish Brown	Present
MS1	Reddish	Absent
MS2	Reddish	Absent
MS3	Dirty Brown	Absent
MS4	Dirty Brown	Absent
MS5	Blackish Brown	Absent

Table 16: Identification of phenols

Samples	1st Methods	2nd Methods
RS	Present	Present
MS1	Absent	Present
MS2	Absent	Present
MS3	Absent	Present
MS4	Absent	Absent
MS5	Absent	Absent

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Table 17: Identification of carbohydrates

Samples	Results
RS	++
MS1	+++
MS2	++
MS3	++
MS4	+
MS5	+

Table 18: Identification of phosphates

Samples	1st Method	2nd Method
RS	Absent	Abent
MS1	Absent	Absent
MS2	Absent	Absent
MS3	Absent	Absent
MS4	Absent	Absent
MS5	Absent	Absent

Table 19: Identification of starch

Samples	Results
RS	Present
MS1	Absent
MS2	Absent
MS3	Absent
MS4	Absent
MS5	Absent

Table 20: Identification of calcium compound

Samples	With CH3COOH	With HCL
RS	+++	+++
MS1	++	++
MS2	++	+++
MS3	+++	+++
MS4	++++	+++
MS5	++	+

Table 21: Identification of iron compound

Samples	Results
RS	Present
MS1	Present
MS2	Present
MS3	Present
MS4	Present
MS5	Present

Table 22: Quantitative determination of total tannins

Samples	% of Total Tannins
RS	3.13
MS1	3.49
MS2	2.25
MS3	2.06
MS4	3.88
MS5	2.32

Table 23: Quantitative determination of total glycosides

Samples	% of Total Glycosides
RS	0.2%
MS1	Absent
MS2	Absent
MS3	Absent
MS4	Absent
MS5	Absent

Fluorescence study

Fluorescence study showed that, when samples were treated with nitrocellulose RS, MS4, MS2 showed, green appearance where as MS1, MS3 were dull green at 254 nm wavelength. At 366nm RS and MS4 showed brown appearance, MS1 and MS2 were light brown and MS3, MS5 were dark brown in appearance. The powered treated with NaOH in methanol under UV 254nm showed green fluorescence for RS, MS1, MS2, MS4 while rest of the samples showed dark green fluorescence. At 366nm

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all the samples showed brown fluorescence. Under UV light when powdered observed as such; at 254nm RS gave yellowish green appearance were as rest of the samples gave green. At 366 nm all samples have brown fluorescence except MS5 which gave dark brown fluorescence (Table 24, 25).

Table 24: Fluorescence characteristics of powdered bark under UV light at 254 nm.

Reagent	RS	MS1	MS2	MS3	MS4	MS5
Powder as such	Yellowish Green	Green	Green	Green	Yellowish Green	Dull Green
Powder mounted in Nitrocellulose	Green	Dull Green	Green	Dull Green	Green	Dull Green
Powder treated with NaOH in Methanol	Green	Green	Green	Dark Green	Green	Dark Green
Powder with NaOH in Methanol and mounted in Nitrocellulose	Green	Green	Green	Green	Green	Green
Powder treated with HCL	Yellowish Green	Green	Green	Green	Yellowish Green	Dull Green

Table 25: Fluorescence characteristics of powdered bark under UV light at 366 nm.

Reagent	RS	MS1	MS2	MS3	MS4	MS5
Powder as such	Brown	Brown	Brown	Brown	Brown	Dark Brown
Powder mounted in Nitrocellulose	Brown	Light Brown	Light Brown	Dark Brown	Brown	Dark Brown
Powder treated with Na OH in Methanol	Brown	Brown	Brown	Brown	Brown	Brown
Powder with Na OH in Methanol and mounted in Nitrocellulose	Brown	Brown	Brown	Brown	Brown	Brown
Powder treated with HCL	Brown	Brown	Brown	Brown	Brown	Dark Brown

Inductive Coupled Plasma Sources for Atomic Emission Spectrophotometry (ICPAES) study

All the 6 samples (R.S. & 5 MS) were analysed for their trace metallic by ICPAES. The RS and MS1 to MS3 were subjected to ICPAES. The RS and MS1 to MS5 were subjected to ICPAES analysis for 17 metals as given in the Table 26. Values were recorded in ppm (µg/g). MS3 shows highest (416 ppm) while RS shows less than 80 ppm of Aluminium. All the samples had less than 10 ppmof Boron &Beryllium. The calcium content was highest in MS4 (43224 ppm) while lowest in MS5 (7544 ppm). Cadmium, Cobalt, Chromium, Manganese, Nickel, Palladium, Valedium and Zinc content in all samples were less than 5,50, 10,50,10,50, 100 and 40 ppm, respectively, while Iron, Magnesium, Manganese, Sodium varied considerably. Magnesium was recorded lowest in RS (648 ppm) and Sodium (33 ppm) Copper was 6 ppmin MS1 and less than 5 ppm in other samples.

Therefore, it can be concluded that the RS shows lowest value for Iron, Sodium and Magnesium. As the Sample is cleared properly shows calcium content (28124 ppm) indicating calcium compounds are present in the RS considerably. As the therapeutic effect is correlated to the presence of Calcium compounds, its concentration present in RS and marketed samples is of great significance.

Table 26: Inductive Coupled Plasma Sources for Atomic Emission Spectrophotometry (ICPAES)

Elements	RS	MS1	MS2	MS3	MS4	MS5
Al	<80	194	172	416	<80	<80
B	<10	<10	<10	<10	<10	<10
Be	<10	<10	<10	<10	<10	<10

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Ca	28124	12024	9924	24824	43224	7544
Cd	<5	<5	<5	<5	<5	<5
Co	<50	<50	<50	<50	<50	<50
Cr	<10	<10	<10	<10	<10	<10
Cu	<5	6	<5	<5	<5	<5
Fe	128	506	311	624	612	362
Mg	698	2456	1749	2679	4049	1319
Mn	10	192	80	28	16	37
Mo	<50	<50	<50	<50	<50	<50
Na	33	171	107	936	185	208
Ni	<10	<10	<10	<10	<10	<10
Pd	<50	<50	<50	<50	<50	<50
V	<100	<100	<100	<100	<100	<100
Zn	<40	<40	<40	<40	<40	<40

1mg/ml solution aliquot was taken for analysis; Values are in PPM (parts per million)

HPTLC STUDY

The HPTLC chromatograms showed no peaks at 366 nm for RS using 2 µl solutions while on application of 4 µl solution, the chromatograph gives 11 spots. The HPTLC chromatograms of marketed samples were recorded and compared with reference sample. As marker compounds were not available, the data obtained from marketed samples cannot be evaluated for phytoconstituents present in the drugs. The quantification of individual phytoconstituents is also not possible without marker compounds. Hence it requires further investigations with respect to phytochemical examination by HPTLC. The finger print data of the reference sample (RS) can be certainly used as standard to compare the quality of the marketed samples. (Table no 27a to 27f)

Table 27a: Substances observed at wavelength 254 nm

Sample	No. of peaks	Total Height	Total Area	No of Peaks	Total Height	Total Area
RS	11	659.8	12693.1	12	704.4	19805.0
MS1	3	379.3	11044.0	7	626.2	18908.0
MS2	12	1403.5	33520.0	11	1670.8	47555.0
MS3	4	472.4	15110.0	7	717.7	23746.4
MS4	6	148.6	3460.9	4	123.1	3188.0
MS5	7	375.6	8499.8	7	676.9	18715.8

Table 27b: Substances observed at wavelength 366nm

Sample	No. of peaks	Total Height	Total Area	No of Peaks	Total Height	Total Area
RS	-	-	-	4	63.1	1431.6
MS1	3	37.8	898.6	5	851.4	18042.8
MS2	8	431.0	10627.9	9	830.4	19982.1
MS3	3	67.9	1515.1	5	884.8	19696.7
MS4	-	-	-	1	12.7	275.9
MS5	1	20.1	428.5	2	156.0	3476.3

Table 27c: Curve observed with R_f 0.46

Sample	R_f	Substance	Wavelength (nm)	Area (mm)
RS	0.46	12	373	535.5
MS2	0.47	12	400	957.7
MS4	0.44	11	372	462.3

Table 27d: Curve observed with R_f0.34

Sample	R_f	Substance	Wavelength (nm)	Area (mm)
RS	0.34	9	350	454.5
MS3	0.36	9	371	2566.7

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Table 27e: Curve Observed with R_f 0.26

Sample	R_f	Substance	Wavelength (nm)	Area (mm)
RS	0.26	7	199	10046.5
MS3	0.23	7	190	9462.8

Table 27f: Curve Observed with R_f 0.17

Sample	R_f	Substance	Wavelength (nm)	Area (mm)
RS	0.17	5	368	129.5
MS5	0.17	5	368	181.6

Water soluble extractive values were nearly same in RS, MS2 and MS3 Alcohol soluble extractive values of RS, MS2 and MS3 Alcohol soluble extractive values of RS was more than other samples. Petroleum ether extractive value of RS was more than MS2 and MS3 whereas less than MS1, MS4, and MS5. Chloroformextractive values of RS and all other samples were almost same. pH of RS and MS4 were acidic while MS1, MS2, MS3 and MS5 had strong acidic pH. Acidic pH indicates sheet Virya.

Tannins were strongly positive in RS where as other showed mild presence of tannins. Steroids, Saponins, Carbohydrates, Calcium and Iron compounds were present in all the samples. Glycosides were only present in RS whereas absent in marketed samples. Phenols were present in RS, MS1, MS2, and MS3 and absent in MS4 and MS5.

To find out authenticity of the Ashoka bark samples available in Mumbai market, were have carried out number of physical and chemical tests. Out of them only few proved to be very significant for deciding genunity of Ashoka bark like Appearance (colour), Microscopic study, Ash values, presence of Steroids, estimation of total Glycosides and Total tannins, HPTLC finger prints. In fluorescence study shows RS have different fluorescent appearance than marketed samples. ICPAES analysis shows varied concentration of elements.

HPTLC finger printing analysis shows reference samples have different peaks and Rf than the marketed samples.

CONCLUSION

The object of the present research work was to Study of *Ashoka* (*Saraca asoca*) bark with special reference to its 5- Samples available in the crude drug market of Mumbai. Previous studies indicate that *Ashoka* bark and its different preparations are very popular and useful in treatment of gynaecological disorders. Various parameters like collection of drug according to methods mentioned in Charaka Samhita, Vimana Sthana Chapter 8, identification by Microscopy, Microscopy, Organoleptic evaluation, Physic-chemical study, Phyto-chemical analysis, Fluorescence study under Ultra-Violet light, ICPAES, HPTLC were done for authentication of the drug. As the demand for the bark is increasing in the market day by day, procuring genuine *Ashoka* bark to be used in the formulation is becoming difficult and leads to adulteration with other bark. Hence, it is very essential to cultivate *Ashoka* on the commercial scale and ensure regular and genuine supply of this drug to the Ayurvedic physician and manufacturers.

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