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Research Article

SUCCESSIVE SOLVENT EXTRACTION AND HPTLC OF STEM BARK OF *ASOKA – SARACA ASOCA* (ROXB.) DE WILDE

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ABSTRACT

Asoka-Saraca asoca (Roxb.) de Wilde, is a medium sized evergreen tree growing in tropical regions. It has been used for various medicinal purposes from the time immemorial. Ample citations about its usage can be elicited from *Veda's, Puranas* and *Samhitas*. Owing to extensive use, lack of cultivation and irrational collection practices it became an endangered drug. It is one among the five endangered plants listed by NMPB. This scarcity of drug in the market eventually led to adulteration. It is one of the severely adulterated drugs next to *Bala - Sida* species. Various pharmacognostical and phytochemical techniques are evolved from time to time to check the adulteration. Due to the sophisticated methodologies used by medicinal plant dealers, these methods fail to check adulteration. Pharmacognostical analysis of sample drug and its powder microscopy serves as an effective method to check adulteration. But it won't serve fruitful when the drug gets adulterated with exhausted samples. In such cases, effective marker compounds of the drug need to be analysed. This can be achieved by analysing successive solvent extractives of test drug and by HPTLC analysis. Here an attempt has been done to analyse the successive solvent extraction and HPTLC of stem bark of *Asoka – Saraca asoca* (Roxb.) de Wilde as an effective methodology to ensure the purity. The successive solvent extraction revealed 1.78%, 0.4%, 13.63% and 27.69% of extractives respectively in petroleum ether, cyclohexane, acetone and methyl alcohol. The qualitative analysis also showed significance difference in the steroids, alkaloids, phenols and flavonoids in each solvent. The results are promising and suggestive of considering these experiments as an effective method to ensure the quality and purity of drug sample.

KEYWORDS: *Asoka*, *Saraca asoca* (Roxb.) de Wilde, Successive Solvent Extraction, HPTLC.

INTRODUCTION

Asoka - Saraca asoca (Roxb.) de Wilde is a medium sized tree found in tropical areas. It is considered as a sacred tree by Hindus and Buddhists. The explanation of word *Asoka* is *"naasti shoko yasmat"* which means the one which relieves or reduces *Soka* (sorrow).[1-3] When *Ravana* captured *Sita*, wife of *Rama,* she became a prisoner in a garden among groves of *Asoka* trees. It is believed that Queen *Maya* gave birth to Siddhartha Gautama, the founder of the Buddhist religion and doctrine of *Nirvana*, under an *Asoka* tree. Hindus revere *Asoka* tree and dedicate it to *Kama Deva*, the god of love. Among *Veda's, Atharvaveda* mentioned *Asoka* as *Agni samana* (similar to fire) owing to its similarity to the *Rupa* (colour) of *Pushpa.* It is explained that if *Homa* (rituals) is performed after having *Asoka Pushpa* with *Madhu* and *Dugdha*, the person will be able to attain *Gandharva pada* (equivalent to celestial people).[4]

Usage of *Asoka* in treatment has been mentioned during *Samhita* period. *Acharya Charaka* mentioned it as *Vedanasthapana mahakashaya* (group of ten pain alleviating drugs), *Susrutacharya* described *Asoka* in *Vrana*sya *Shashti Upakrama* (60 treatment principles for wounds). Detailed description of *Asoka* is also available from various *Nighantu*s. In current treatment practice, its stem bark has been widely used as an effective remedy for dysmenorrhea.[5,6]

Due to extensive use and irrational collection practices there is a drastic decrease in the availability of drug. Eventually it resulted in the adulteration of drug in the market. The stem bark of *Asoka* has been widely adulterated with the bark of *Polyalthia longifolia*as it possesses the name *Asoka* in Tamil. Often it is adulterated with *Rohitaka* bark (*Afanamexis polystakis*) and bark of *Sicalpinea pulchirena.*[7]

Adulteration of market samples is one of the greatest drawbacks in promotion of Ayurvedic pharmaceutical industry. Many research

methodologies have been contributed to check adulterations and to authenticate the drug samples. It has been found that the adverse drug event responses are occurring greatly due to the presence of unintended drug in a combination. Medicinal plant dealers have discovered many scientific methods to create adulteration of such a high quality so that it is difficult to trace those adulterations.[8]

Pharmacognostical analysis is one of the basic methods to ensure the identity of a drug. In this context often, it fails owing to the close similarity of dry bark samples. As a 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 procedures and requires more time and money. So, here an attempt has been done to analyse whether successive solvent extraction can be an effective methodology to analyse the specific constituents in different extracts. Also HPTLC studies were done to compare the quality of present sample with that of API standards.

MATERIALS AND METHODS

Collection of Test Drug

The study drug, stem bark of *Asoka- Saraca asoca* (Roxb.) de Wilde. was collected from its natural habitat of Perumbavur, Ernakulam district. Sample was pharmacognostically identified in the Pharmacognosy Lab, Department of Dravyaguna vijnanam, Government Ayurveda College, Tripunithura. Collected samples were washed with water thoroughly to remove physical impurities like soil, mud etc., and shade dried, powdered and kept in airtight containers.

Fig: 1 Stem of *Asoka – Saraca asoca* (Roxb.) de Wilde.

Fig:2 Leaves of *Asoka – Saraca asoca* (Roxb.) de Wilde.

Fig:3 Inflorescence of *Asoka – Saraca asoca* (Roxb.) de Wilde.

Fig: 4 Pods of *Asoka – Saraca asoca* (Roxb.) de Wilde.

Fig:1&2 - Stem bark of *Asoka – Saraca asoca* (Roxb.) de Wilde.

Fig:3 Powder of stem bark of *Asoka – Saraca asoca* (Roxb.) de Wilde.

Successive Solvent Extraction

Extraction is a commonly employed technique for the removal of active substances from crude drug, involving the use of different solvents. 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.

Procedure

10g of accurately weighed powder of stem bark of *Asoka - Saraca asoca* (Roxb). de Wilde was taken in thimbles and was put in Soxhlet extractor and placed on top of a well-supported Round bottom flask containing Petroleum ether with few glass beads. A reflux condenser was properly connected to a running tap water and was placed on top of Soxhlet extractor. The flasks were then heated in a water bath continuously to get the solvent boiled and its vapour reaches the Soxhlet extractor, after condensation, passes through the thimbles containing drugs. When the condensed solution reaches the top of the small siphon tube, the solvent solution flows back through the narrow tube and returns to the round bottom flasks where the extracted materials gets accumulated.

Extraction was continued till the solvent in the siphon tube was colourless. The system could stand and cool to room temperature. The solvent in round bottom flasks was then evaporated and the concentrated extractive was transferred into pre-weighed 100ml beaker and evaporated to dryness over a water bath and get dried in desiccators. The extractive obtained with solvents was accurately weighed and recorded for each solvent. The colour and consistency of the extracts was noted. Each time before doing the extraction with next solvent, the extracted material was dried in hot air oven below 50 degrees Celsius. The whole process was repeated with solvents cyclohexane, acetone and alcohol successively. Each time the extractive obtained was weighed and the percentage of extract was calculated with reference to air dried samples of powder of stem bark of *Asoka – Saraca asoca* (Roxb.) de Wilde.

The extractives obtained by successive solvent extraction were analysed qualitatively for identification of various plant constituents.

Qualitative Analysis of Successive Solvent Extractives

1) Steroids

To 2.0ml of the solution of chloroform extracts of powder of stem bark of *Asoka - Saraca asoca* (Roxb.) de Wilde was taken in test tube, 1.0ml of concentrated Sulphuric acid was added carefully along the sides of the test tube. A red colour was produced in the chloroform layer indicates presence of steroids.

2) Flavonoids

To 0.5ml of the solution of alcoholic extract of powder of stem bark of *Asoka - Saraca asoca* (Roxb.) de Wilde was taken in test tubes, 5-10 drops of dilute hydrochloric acid was added and a small piece of magnesium were added and the solution was boiled for few minutes. Presence of pink colour indicates presence of flavonoids.

3) Alkaloids

a. To 0.5ml of alcoholic extract of powder of stem bark of *Asoka - Saraca asoca* (Roxb.) de Wilde was taken in test tube, 2.0ml of Hydrochloric acid solution was added. To this acidic medium, 1.0ml of Dragendroff"s reagent was added. An orange - red precipitate produced immediately indicated the presence of alkaloids.

b. To 10ml of the solution of alcoholic extracts of powder of stem bark of *Asoka - Saraca asoca* (Roxb.) de Wilde was taken in test tubes, a few drops of Meyer's reagent was added. Formation of white or pale precipitate indicated the presence of alkaloids.

4) Phenols

i. Ferric Chloride test

To 1.0 ml of the solution of the alcoholic extract of powder of stem bark of *Asoka* (*Saraca asoca* (Roxb). de Wilde) was taken in test tubes, 2.0ml of distilled water was added followed by addition of a few drops of 10% aqueous ferric chloride solution. Formation of blue or green colour indicated the presence of phenols.

ii. Lead acetate test

1.0ml of the solution of the alcoholic extracts of powder of stem bark of *Asoka* - *Saraca asoca* (Roxb.) de Wilde was taken in test tube. 5ml distilled water was added followed by few drops of 1% aqueous solution of lead acetate. The formation of yellow precipitate in test tubes indicated the presence of phenols.

HPTLC

Procedure

1g powder of stem bark of *Asoka* - *Saraca asoca* (Roxb.) de Wilde was weighed, extracted with 10ml methanol and spotted as 15 micro-liter. The stationary phase was Merk, 1.05554.0007, TLC Silica gel 60 F254, 10x10cm Aluminium sheet. The mobile phase was Toluene: Ethyl acetate: Formic acid: Methanol (7:5:1:0.5). 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.

RESULTS

Results are tabulated as below.

Results of successive solvent extraction

Table 1: Results of successive solvent extraction

Sl.No	Solvent	Percentage of extractive value
1.	Petroleum ether	1.78%
2.	Cyclohexane	0.4%
3.	Acetone	13.63%
4.	Methyl alcohol	27.69%

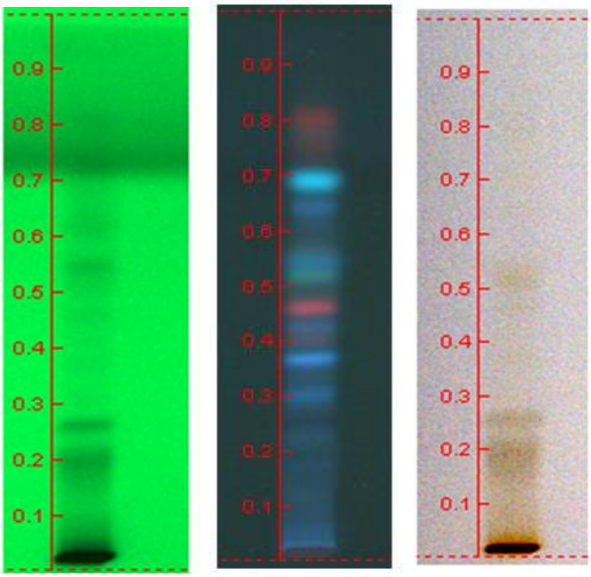
Results of qualitative analysis of successive solvent extraction

Table 2: Results of qualitative analysis of successive solvent extractives

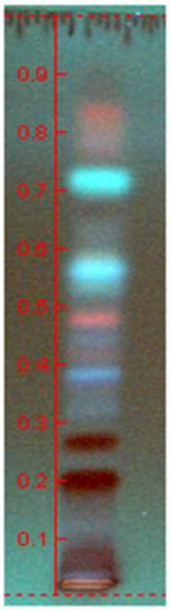
Sl No:	Extract	Steroids	Alkaloids	Flavonoids	Phenols
1	Petroleum ether	+	+	-	-
2	Cyclohexane	-	++	-	-
3	Acetone	+	-	+	++
4	Alcohol	+	+	+	+

Results of HPTLC analysis are given below.

At 254nm At 366nm At white light



Derivatization at 366nm



Derivatization at white light

Rf values and percentage areas at 254nm & 366nm are tabulated below

Table 3: Rf value & percentage of area at 254nm

Peak No.	Rf Value	Area(AU)	% Area(AU)
1	0.04	362.6	0.89
2	0.19	9825.3	24.23
3	0.26	3140.4	7.74
4	0.30	186.2	0.46
5	0.37	363.4	0.90
6	0.46	572.2	1.41
7	0.55	4880.2	12.03
8	0.62	1617.3	3.99
9	0.74	19597.0	48.32
10	0.94	13.6	0.03

Total peak No – 10

Total area – 40558.2 (AU)

Table 4: Rf value & percentage of area at 366nm

Peak No.	Rf Value	Area(AU)	% Area(AU)
1	0.19	7541.6	26.93
2	0.26	1118.7	3.99
3	0.30	187.6	0.67
4	0.38	513.1	1.83
5	0.47	422.6	1.51
6	0.55	15425.6	55.09
7	0.65	104.0	0.38
8	0.71	2124.3	7.59
9	0.80	379.2	1.35
10	0.94	185.5	0.66

Total peak no – 10

Total area –28002.2 (AU)

DISCUSSION

The extractive values of powder of stem bark of *Asoka- Saraca asoca* (Roxb.) de Wilde. during successive solvent extraction using solvents petroleum ether, cyclohexane, acetone and methyl alcohol are respectively 1.78%, 0.4%, 13.63% and 27.69%. The availability of drug during the extraction increases with the increase of polarity of solvent. This shows the presence of ionizable compounds in the drug. Qualitative analysis of extractive of the drug in petroleum ether revealed the presence of steroids and alkaloids. The analysis of extractives using cyclohexane revealed the presence of alkaloids. The analysis of extractives of acetone revealed presence of steroids, flavonoids and phenols. The analysis of extractives in alcohols showed the presence of steroids, alkaloids, flavonoids and phenols.

HPTLC analysis of powder of stem bark of *Asoka- Saraca asoca* (Roxb.) de Wilde. in ethanolic extract reveals 10 peaks with total area of 40558.2 A.U at 254nm. Among them the two major peaks were seen at Rf 0.74 with an area 19597.0 A.U (48.32%) and Rf 0.55 with an area 4880.2 A.U (12.03%). At 366nm, HPTLC analysis reveals 10 peaks with total area of 28002.2 A.U. Among them the two major peaks were seen at Rf 0.19 with an area 7541.6 A.U (26.93%) and Rf 0.55 with an area 15425.6 A.U (55.09%).

CONCLUSION

The successive solvent extractions of powder of stem bark of *Asoka- Saraca asoca* (Roxb.) de Wilde. is an innovative technique to distinguish the adulteration of drug sample. It is cost effective and don't need much technical expatriations. Also, HPTLC studies reveal the details about marker chemical constituents in genuine sample of test drug.

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REFERENCES

1. Sir Monnier & Williams. Sanskrit English dictionary, 16th reprint, 2011, Delhi, Motilal Barasidass Publishers Private Limited, p:113.
2. Raja Radha Kanta Deva. Editor, Sabdakalpadrumam, Varanasi, Chaukambha Sanskrit Series, p:137.
3. Pandit Haragovinda Sastri. Editor, Amarakosham of Amarasimha with Ramasrami Commentary of Bhanuji Dikshita, Reprint 2012, Varanasi, Chaukambha Sanskri Series, 2nd kandham, Vanoushadhi varga, p:202.
4. Shastry J L N. Illustrated Dravyagunavijnana, Study of Essential Medicinal Plants in Ayurveda, Varanasi, Choukambha Orientalia, Vol.1, p:13.
5. Sharma R K and Dash B. Charaka Samhita: English translation and critical exposition based on Chakrapanidatta's Ayurvedadipika. Chowkamba Sanskrit Series, Varanasi, 2014, Vol 1: p.100.
6. Sreekantha Murthy. Translator, Illustrated Susruta Samhita, Reprint 2014, Varanasi, Chaukambha Orientalia, Vol:2, p:20.
7. Vaidya Bapalal. Some Controversial Drugs in Indian Medicine, Chaukambha Orientalia, Reprint: 2014; p:25-31.
8. Om Prakash et al, Adulteration and Substitution in Indian Medicinal Plants: An Overview, Journal of Medicinal Plants Studies, 2013, Volume: 1, Issue: 4; page: 127 – 132.

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