IR and $^1$H-NMR based differentiation of stem bark of *Saraca asoca* from its most commonly used adulterant *Polyalthia longifolia*

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The bark of the *Saraca asoca* (Roxb.) de Wilde plant is used widely in treating various diseases, mainly uterine disorders. However, owing to its shortage, *S. asoca* formulations are now being adulterated with the barks of other plants, *Polyalthia longifolia* (Sonn.) Thwaites being the most common. Fourier-transform infra-red spectroscopy (FTIR) and $^1$H Nuclear Magnetic Resonance (NMR) spectroscopy-based effective, feasible and fast methods can be used for differentiating *S. asoca* bark from that of *P. longifolia*. The unique chemical shift values of different chemical constituents in methanolic, ethyl acetate and hexane extracts of barks of both plants were qualitatively identified and compared, and a new method of differentiating the adulterant from *Saraca asoca* bark has been established.

**Keywords:** $^1$H-NMR, FT-IR, Herbal adulteration, *Polyalthia longifolia* (Sonn.) Thwaites, *Saraca asoca* (Roxb.) de Wilde

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**Introduction**

*Saraca asoca* (Roxb.) De Wilde, one of the most important medicinal plants, is a medium-sized, evergreen tree belonging to the family Caesalpinaceae. The tree, popularly known as “True Ashoka”, is widely distributed throughout India, especially in West Bengal, Kerala and the Himalayas, with North-west India being the only exception. Ashoka stem bark is used in Ayurveda to treat dyspepsia, fever, visceromegaly, ulcerations, blood disorders and, most significantly, menstrual irregularities, uterine bleeding, and menorrhagia. The drug is also reported as having astringent, demulcent, anthelmintic, alexiteric, emollient and refreshing properties. Widely known and popular alcoholic Ayurvedic formulations “Ashokarishta” and “Ashokaghritha” are prepared with Ashoka bark as the principal ingredient. The aqueous and alcoholic extracts showed antioxidant potential and protection against lead-induced toxicity in HepG2 and HEK293 cell lines. The ethanolic extract also showed shigelloidal activity against *Shigella dysentriae*. Kumar V developed the RAPD-derived SCAR marker for differentiation and identification of *S. asoca* from widely used adulterant.

*S. asoca* is listed as an ingredient in more than 50 preparations used in Ayurveda. Of late, there has been increasing difficulty in fulfilling the ever-rising demand for *S. asoca* bark due to the inherent slow growth rate, poor tree yield, and difficulties in large-scale commercial cultivation. One reason responsible for the dwindling availability of the stem bark of Ashoka is overharvesting caused by the high demand for this tree. Due to the destructive extraction process and absence of a scientific and organised cultivation system, this extremely important therapeutically beneficial tree is on the verge of extinction. The plant is red-listed in the vulnerable category and has been reported to be endangered.

Adulterating and substituting endangered or rare raw materials are frequent in the herbal trade industry. In order to meet the excessively large demand for Ashoka bark in the face of acute shortage, traders are nowadays admixing the bark of other trees in *S. asoca* formulation, and the adulterated products do not possess the claimed therapeutic effects of Ashoka. The most prevalently used adulterant is the bark of a fast-growing plant, *Polyalthia longifolia* (Sonn.) Thwaites, which has gained the name of “False Ashoka” due to its rampant use as an adulterant. It belongs to the family Annonaceae and is native to India and Sri Lanka and cultivated in Bhutan, China, and other tropical countries. Although...
it has shown pharmacological effects in treating diarrhoea and epilepsy, its presence as an adulterant in Ashoka formulations diminishes the efficacy of the formulations. It is very difficult to differentiate the dried stem bark of *P. longifolia* from the crude drug of *S. asoca* by the naked eye, making its use as an adulterant easier. Several methods have been developed and validated to differentiate *P. longifolia* bark from *S. asoca*. The methods include phytochemical markers and differentiation through anatomical, biochemical, and chromatographic study of the bark of two plants. HPLC-ESI/MS characterisation of catechin fractions has also been employed to identify the presence of adulterant(s) in *S. asoca* sample. Inter Simple Sequence Repeat (ISSR)-based Hierarchical Cluster Analysis was carried out by DNA extraction, amplifications of ISSR and followed by data analysis to develop a unique way for differentiating crude drug from the adulterant. Categorisation of *S. asoca* and *P. longifolia* extracts is also executed using chemical and genetic fingerprinting, in association with multivariate statistical analysis using High-Performance Thin Layer Chromatography (HPTLC) analysis and Amplified Fragment Length Polymorphism (AFLP). Though these methods have been successful in differentiating *P. longifolia* bark from *S. asoca*, they have certain disadvantages. Genetic fingerprinting is a long, tedious and time-consuming process. It is important to use a highly purified and high molecular weight DNA sample, which is hard to get and thus makes the process less feasible. Chromatographic methods like HPLC and HPTLC require authentic reference markers, which are generally difficult to isolate and purify; HPLC often requires elaborate sample preparation procedures and method development, while HPTLC offers poor resolution compared to HPLC.

Therefore, based on these facts and the need, we report here a fast and reliable method for the identification and differentiation of the adulterant of “False Ashoka” from a sample of “True Ashoka” using Fourier Transform-Infra Red (FTIR) and Nuclear Magnetic Resonance (NMR) Spectroscopy. NMR can analyse many molecules in a mixture in a simple and fast acquisition process. IR spectroscopy is also an efficient analytical technique where results can be obtained almost instantaneously, speeding up the process. The work aimed to develop a promising analytical approach for identifying the adulteration in the bark of *S. asoca* by *P. longifolia*.

Materials and Methods

**Procurement of plant material**

Stem barks of *S. asoca* (Voucher number: NIP-M-634) and *P. longifolia* (Voucher number: NIP-M-635) collected in July 2022, stem bark from young trees of *S. asoca* (Voucher number: NIP-M-2054) and *P. longifolia* (Voucher number: NIP-M-2055), stem bark from mature trees of *S. asoca* (Voucher number: NIP-M-2056) and *P. longifolia* (Voucher number: NIP-M-2057) were collected in September 2023 from the campus area of National Institute of Pharmaceutical Education and Research, S. A.S. Nagar, India. Stem barks of *S. asoca* (Voucher number: NIP-M-2058) and *P. longifolia* (Voucher number: NIP-M-2059) were also procured in October 2023 from Durgapur, West Bengal. All the plant samples were authenticated by Dr. Alok Goyal, Botanist, Department of Natural Products, NIPER.

**Chemicals**

CDCl$_3$ with added internal standard, TMS (0.03%), and CD$_3$OD used as NMR solvents were purchased from SYNMR Chemicals Pvt. Ltd, Bangalore, India. The solvents used to extract bark powder (methanol, ethyl acetate, and hexane) were first distilled and then used for extraction.

**Instruments**

The FTIR spectra were recorded on a Perkin Elmer spectrophotometer (Universal ATR Sampling Accessory)/Shimadzu IRTracer-100, and the $^1$H NMR spectra were recorded on a 600MHz (JEOL JNM-ECZ600R, Japan) NMR instrument. The extracts were concentrated on a rotary evaporator (Buchi).

**Preparation of the extract**

The barks were ground into powdered form using a pulveriser. 100 g of powdered material (*S. asoca* and *P. longifolia* each) were refluxed with methanol for 1 hour. This whole process was repeated in triplicate. The combined extract was filtered using filter paper and concentrated using a rotary evaporator. The extracts obtained were weighed. The percentage yield was 18.48 and 13.68% for *S. asoca* and *P. longifolia* extracts, respectively. Hexane and ethyl acetate extracts were similarly prepared from 50 g each of *S. asoca* and *P. longifolia* barks. The percentage yield was 0.216 and 2.28% of *S. asoca* and *P. longifolia* in hexane and 0.76 and 4.98% in ethyl acetate, respectively.

The FTIR spectra were recorded by dissolving about 10 mg of extract in dichloromethane as a...
solvent or neat. For $^1$H NMR, the spectra were recorded by dissolving 15 mg of extract in 0.6 mL of CDCl$_3$ or CD$_3$OD. All chemical shifts were assigned relative to TMS as an internal standard. All the extracts from *S. asoca* and *P. longifolia* were mixed in equal amounts and dissolved in the solvent to measure the $^1$HNMR spectra of the extract mixture.

**Results and Discussion**

The major and minor compounds reported in these two plant barks were identified from the literature to study the chemistry of these plants and to see if any major differences can be identified in the IR and NMR spectra of the crude extracts derived from these plants. It is observed that these plants’ stem bark exhibited entirely distinct chemical profiles.

*S. asoca* bark is reported to contain mainly condensed tannins like catechin (1), gallocatechin (2), epicatechin (3), epigallocatechin (4), gallocatechingallate (5), catechingallate (6), epigallocatechingallate (7), epicatechingallate (8), procyanidin B2 (9), 11'-deoxyproanthocyanidin B (10), 3'-deoxycatechin-3-O-$\alpha$-L-rhamnopyranoside (11), 3'-deoxycatechin-3-O-$\beta$-L-glucopyranoside (12), lignan glycosides like lyoniside (13), nudiposide (14), schizandriside (15), icariside E3 (16), 5-methoxy-9$\beta$-D-xylopyranosyl(-)-isorariciresinol (17), sterols like lupeol (18), ursolic acid (19), $\beta$-sitosterol (20), stigmasterol (21), campesterol (22), cyclitol pinitol (23), flavanoids apigenin (24), quercetin (25), leucocyanidin (26) and leucopelargonidin (27). The structures of compounds reported in the stem bark of *S. asoca* are shown in Fig. 1.


![Fig. 1 — Phytochemical constituents of *S. asoca* bark (1-27).](image-url)
ent-halima-5(10),13E-dien-15-oic acid (39), ent-halima-1(10),13E-dien-15-oic acid (40), 16-oxocleroda-3,13E-dien-15-oic acid (41), 16-oxo-ent-halima-5(10)-13E-dien-15-oic acid (42), cleroda-3,13-dien-16,15-olide (43), cleroda-4(18),13-dien-16, 15-olide (44), ent-halima-5(10),13E-dien-16,15-olide (45), ent-halima-1(10),13E-dien-16,15-olide (46) and γ-methylbutenolide (47). The structures are shown in Fig. 2.

A comparison of the structures and reported NMR data of the above compounds suggested that it should be possible to differentiate the barks of these two plants from each other or in a mixture using IR and 1HNMR spectroscopy. Numerous literature reports on using NMR and IR spectroscopy for identifying and quantitating complex plant extracts containing multiple components.

FTIR based differentiation

The transmittance vs wavenumber spectra of methanolic, hexane and ethyl acetate extracts of S. asoca and P. longifolia were recorded. It was observed that while most of the bands of both extracts were almost similar, some prominent bands could be used for identifying the extracts. A distinct carbonyl carbon band at 1713.26 cm\(^{-1}\) could be seen in P. longifolia, while no such band was found in S. asoca. Another prominent band around 1605 cm\(^{-1}\) could be seen in both plants. Distinct bands at 1437.26 and 1517.17 cm\(^{-1}\) could be seen in the IR spectrum of S. asoca. These prominent unique bands can be perceived as discernible characteristics to differentiate the bark extracts of these plants. The usability of these bands was further confirmed by recording IR spectra of different samples of these two plants collected at different ages, at different maturity stages, and in different years, as shown in the overlaid spectra (Fig. 3). All samples of P. longifolia invariably showed the carbonyl band.

1HNMR based differentiation

Methanolic extract

The 1HNMR spectra of S. asoca and P. longifolia could be easily differentiated by analysing the differences in the signal patterns of both plants. P. longifolia contains clerodane diterpenes as a distinct class of compounds. Many of these compounds contain α, β-unsaturated butyrolactone, an aldehydic group or a carboxylic group. The signals from these...
Fig. 3 — a) FTIR-based comparison of methanolic extracts of *S. asoca* and *P. longifolia*; b) FTIR overlay of hexane extract of *P. longifolia* and *S. asoca* bark. Sample 1—*S. asoca* bark procured from West Bengal. Sample 2—*S. asoca* bark (young tree) procured from NIPER, Mohali. Sample 3 *S. asoca* bark procured from NIPER, Mohali (mature tree). Sample 6-*P. longifolia* bark (mature tree) procured from NIPER, Mohali. Sample 7-*P. longifolia* (young tree) bark procured from NIPER, Mohali. Sample 8-*P. longifolia* bark procured from West Bengal; and c) Overlay of ethyl acetate extract of *P. longifolia* and *S. asoca* bark. Sample codes are the same as in Fig. 3b.
groups can be taken as distinguishing features to confirm the presence of this adulterant in S. asoca bark. Prominent \(^1\)H NMR signals of lignan glycosides and catechins generally appear in the region \(\delta 3.3\) to 3.8 and 6.6 and 6.8 ppm\(^{25-29}\).

The methanolic extract of S. asoca also showed \(^1\)H NMR signals in the above chemical shift regions in the range of \(\delta 6.6\) and 6.8 for catechins and 3.3 to 3.8 for lignan glycosides (13-17). The \(^1\)HNMR spectrum showed signals at \(\delta 4.02\) (H-3), 5.91 (H-8) and 5.94 (H-6) assigned to compound 2 or related catechins\(^{26}\). A doublet at \(\delta 4.47\) (H-2) was assigned to compound 26\(^{27}\). A broad singlet at \(\delta 4.57\) was assigned to H-2 of compound 4, and two singlet signals at \(\delta 5.09\) and 5.10 could be assigned to H-2 of compounds 6 and 8, respectively\(^{28}\). A doublet at \(\delta 6.97\) was assigned to H-2' of compound 6. In contrast, the \(^1\)HNMR spectrum of the methanolic extract of P. longifolia showed signals related to clerodane diterpenes; prominent signals at \(\delta 9.39\) and 9.38 (H-16) were assigned to the aldehyde group of compounds 28 and 29. The other signals at \(\delta 6.71\) (H-12), 5.18 (H-3), and 2.34 (H-11) confirmed the presence of compound 28\(^{30}\). A broad singlet at \(\delta 5.64\) assigned to H-14 indicated the presence of compounds 30, 31, 39, 40.

The signals in the range of \(\delta 9.3\) to 9.5 from aldehyde groups are absent in the spectrum of S. asoca, marking a major difference in the identification of adulterants. Another stark contrasting feature of the two spectra is the presence of many signals in the range of \(\delta 0.5-2\) in P. longifolia due to many alkyl groups, which is not found in the methanolic extract of S. asoca. Fig. 4 shows overlapped spectra of methanolic extracts of S. asoca and P. longifolia, where the characteristic chemistry of both plants is clearly reflected in the spectra.

**Hexane extract**

The difference between the spectra of the two extracts can be observed in the range of \(\delta 2.00\) to 5.10. In the \(^1\)HNMR spectrum of hexane extract of S. asoca, olefinic methylene signals of compound 18 were observed at 4.56 and 4.68 (H-29)\(^{31}\). The signal at \(\delta 3.52\) (H-3) and at \(\delta 5.33\) (H-6) was assigned to 20\(^{32}\). Signals of sterols could be observed at \(\delta 3.20\) (H-3) for compounds 18 and 22\(^{31}\). The chemical shift range from \(\delta 4.5\) to 7.0 is unique in the P. longifolia spectrum. Signals of multiple compounds can be observed at \(\delta 5.67\) (H-14), indicating the presence of compounds 30, 31, 39 and 40, at 6.01 for compounds 32, 37, 38 (H-16)\(^{17}\). Broad signals at \(\delta 5.34\) (H-1) were assigned to 40 and 46, and 5.83 (H-14) to 32, 37, 38, 43-46\(^{17}\). A triplet at \(\delta 6.87\) identified compound 28\(^{33}\). Signals of S. asoca and P. longifolia are overlapped at positions of 3.25 due to the presence of compounds 20 (H-3) and 28 (H-14)\(^{32}\), 4.51 due to the presence of compound 18 (H-29) and 45 (H-16).

![Fig. 4 — \(^1\)H-NMR spectra of methanol extracts of S. asoca and P. longifolia bark.](image-url)
Both spectra feature a large number of signals in the $\delta$ 0.5-2 range due to the presence of a large number of alkyl groups of sterols and terpenes in *S. asoca* and *P. longifolia*, respectively. Fig. 5a shows the $^1$H NMR spectra of two extracts where the assigned signals to individual compounds are marked. Fig. 5b shows overlaid spectra of hexane extracts of the stem bark of both the plants collected from different regions and at different maturity stages. The overlaid spectra clearly show that the characteristic signals used to differentiate between stem bark are present in all samples, reflecting the different chemical profiles of the two plants. The distinguishing signals of the two plants are marked with arrows.

**Ethyl acetate extract**

The differences in chemical profiles were also clearly discernible from the ethyl acetate spectra of the bark of both plants. The $^1$HNMR signals in the range of $\delta$ 9.3 to 9.5 in the *P. longifolia* extract

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![Image of spectra](image-url)
indicated the presence of aldehydic clerodane terpenes containing an aldehyde group, which was absent in *S. asoca* extract. Although *S. asoca* spectra feature a signal at δ 3.65 and a doublet at 4.29 for H-3 of compounds 1 and 3, respectively, a broad singlet was observed at δ 5.12 for H-4 of compounds 9 and 10. *P. longifolia* ethyl acetate extract spectrum was much more complex in the range of δ 3 to 7 due to the presence of multiple clerodane diterpenes; compound 28 with its signals at 9.53 (H-16) and 6.81 (H-12); a hump at δ 4.51 for H-18 of compounds 29, 31, 37, and 44, a broad singlet at δ 4.74 for H-16 of 43-46. As shown in Fig. 6a, signals from several other compounds were also characteristic of *P. longifolia*. Both spectra showed many signals in δ 0.5-2 due to the presence of alkyl groups (Fig. 6a). The overlaid spectra of different samples, as shown in Fig. 6b, illustrate the usability of specified signals.

### Extract Mixtures

From the 1H-NMR spectrum of combined hexane extracts of *S. asoca* and *P. longifolia*, signals of compounds present in both the plant barks could be

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Fig. 6 — a) 1H-NMR spectra of ethyl acetate extracts of *S. asoca* and *P. longifolia* bark; and b) Overlay of ethyl acetate extract of *P. longifolia* and *S. asoca* bark. Sample coding is the same as in Fig. 5b.
observed. Signals with their chemical shifts resembling sterols and catechins of *S. asoca* and clerodane diterpenes of *P. longifolia* can be observed. Similarly, separate signals could be seen in the 

$^1$H-NMR spectrum of mixed ethyl acetate extracts of both plants (Figs. 7 and 8).

The adulteration of *S. asoca* with *P. longifolia* is a serious issue as it affects the quality of herbal/
traditional formulations of *S. asoca*. It is important to find fast and reliable methods to detect the presence of this adulterant. A visual inspection of IR and NMR spectra of the stem bark of these two plants can readily indicate the presence of an adulterant. The characteristic $^1$H NMR signals from the aldehydic or carboxylic groups indicate adulteration with *P. longifolia*.

**Conclusion**

We have established simple IR and $^1$HNMR methods for differentiating between the bark of *S. asoca* and its most commonly used adulterant, *P. longifolia*. The above studies also suggest that the chemical profiles of the two plants are very different; therefore, preparations using *P. longifolia* instead of *S. asoca* bark may not have the same efficacy. The developed method has potential applications in quality control of herbal formulations. Both NMR and IR can be routinely used for detection of adulterants or substitutes in herbal materials, where the chemistry of plants is different as in the case of *S. asoca* and *P. longifolia*. qNMR has emerged as a powerful technique for quantitative analysis of plant extracts and herbal formulations.

**Conflict of interest**

The authors declare no conflict of Interest.

**References**


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