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Quality assessment evaluation of the market samples of important ayurvedic drug asoka bark

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Abstract

***S**araca asoca (*Roxb.) de Wilde, is locally known as *Sita asok* and is grown as an ornamental tree throughout the country. But bark of asoka tree is an important herbal raw drug used in gynaecological problems like uterine bleeding associated with fibroids. It is a major constituent of the ayurvedic medicines "asokarishtam" and "asokagritham"used for the treatment of gynaecological disorders. The increasing demand of the bark along with less availability has led to its widespread adulteration. It is widely adulterated with the bark of *Polyalthia longifolia*, a well known avenue tree available in plenty. Since adulteration of herbal drugs has clinically relevant effects, an attempt has been made to develop an easy method to detect the adulteration in market samples of the raw drug asoka bark and to develop specific chemical fingerprints of the genuine bark by TLC (Thin Layer Chromatography). Petroleum ether extracts of the stem barks of asoka and polyalthia were found best suitable to develop good chromatograms. These specific chromatograms proved efficient in detecting adulteration in 22 market samples analysed.

Key words: Adulteration, chemical fingerprinting, *Saraca asoca*, *Polyalthia longifolia*, Thin Layer Chromatography (TLC).

Introduction

***S**araca asoca (*Roxb.) de Wilde. commonly known as *Sita asok* (Figure 1) is a sacred tree of India. This redlisted medicinal plant of the Western Ghats belongs to the family, *Caesalpiniaceae* (Nayar *et al.*, 1990). Bark of *Saraca asoca*, rich in tannin is the medicinal part, widely used in the treatment of gynaecological disorders. Tannins provide the main astringent action. Asoka bark is the major ingredient in famous ayurvedic formulations like "ashokarishtam" and "asokagritham", used in treating gynaec ailments as excessive uterine bleeding and irregular menstruation (Nadkarni, 2005). It is also used for treating leucorrhoea, dyspepsia, blood disorders, tumours, indigestion *etc*. (Warrier, 1996). It was estimated that the domestic demand of the bark of *Saraca asoca* was 5332 metric tonnes during 1999-2000 and the estimated demand level is more than 15,000 tonnes for the year 2007-11 (Study Report of The Ministry of Health, 2001-02). The gap between increasing demand and low availability is met with obviously by adulteration. Survey studies have reported that the bark of asoka is widely adulterated with the bark of polyalthia (Parvathi Menon, 2002) which is abundantly available. External appearance of the bark of asoka and polyalthia are so similar that even an expert finds it difficult to detect the adulteration just by visual screening. *Polyalthia longifolia* is a beautiful lofty evergreen tree found in India and Sri Lanka, belonging to the family, *Annonaceae* (Figure. 2). It is the common ornamental street tree, which can very well combat noise pollution. Active ingredients that contribute to the medicinal property of asoka are phenols and tannins whereas that of polyalthia are alkaloids (Wealth of India, 1998). Substituting asoka with polyalthia may not be effective in treating gynaecological disorders or it may rather lead to some health hazards whose symptoms may develop only later.

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Adulteration of herbal products has clinically relevant effects (Sperl *et al.*, 1995 and Nelson *et al.*, 1995).

The present study was conducted to standardize a simple, efficient and easy chemical method to test and ensure the quality of raw drug, *Saraca asoca* bark available in the market and, thus, to detect the adulteration, if any.

Chromatographic fingerprinting has been in use for a long time for evaluation of herbal drugs on a phytochemical basis (Li *et al.*,1998). Thin-layer chromatography can be successfully used for standardization and to control the quality of both the raw material and the finished products also . Efficacy of drugs can be evaluated by the use of several properties and characteristics of the chromatogram, like distance of migration of the compounds resolved (R_f), the spots as observed with the naked eye, as examined under UV illumination and the response to several reagents during derivatization. Such a profile is distinct and forms a benchmark of the drug, especially when identities of active principles are not known or when chemical markers are not available for analysis. As a part of the comparative analysis of the stem barks of asoka and polyalthia, we report simple, specific, sensitive and reproducible method for detecting adulteration in asoka using TLC. We tried different combinations of organic solvents in varying proportions and different colour reagents to develop good fingerprints which can be used for differentiation of asoka from polyalthia. Out of these, the best suitable systems was selected and using this tool, adulteration screening was done in 22 market samples, collected from various markets of Kerala and the results are presented in this paper.

Materials and Methods

Stem barks of *Saraca asoca* (Roxb.) de Wilde and *Polyalthia longifolia* (Sonn.) Thwaites were collected from College of Horticulture, Kerala Agricultural University, Thrissur, Kerala, India. The samples were shade dried and powdered. Five gram fine powder of each of the samples was refluxed with 50 ml petroleum ether overnight. These extracts were cooled to room temperature, filtered, concentrated by evaporation under vacuum and was used for the chemical fingerprinting studies, using chromato-graphic techniques. Pre-coated flourescent silica gel 60 F_{254} plates were used as the stationary phase and benzene: ethyl acetate (9.7:0.3) as mobile phase. The plates were developed up to a length of 8 cm in a CAMAG glass twin trough chamber (10 x 10 cm), previously saturated with the solvent systems for 15 minutes. Solvent systems suitable for separation of components were standardized by trying different combinations of organic solvents in varying proportions. After removal from the mobile phase, the plates were left to dry and sprayed with antimony trichloride TLC reagent. After drying in a hot air oven for 5 minutes at 90°C viewed under UV-365 nm. The nature of bands developed and their R_f values were recorded. Twenty two asoka bark market samples were collected from various markets of Kerala and dried and powdered. Petroleum ether extracts were prepared and used for developing TLC chromatogram. These chromatograms were compared with the TLC chromatograms of genuine asoka and polyalthia bark samples.

Results and Discussion

A good chromatographic fingerprint was obtained for the petroleum ether extracts of *Saraca asoca* and *Polyalthia longifolia* when developed in benzene: ethyl acetate, 9.3:0.7 (v/v) and derivatized by spraying with the TLC colour reagent antimony trichloride and heated at 100°C for 5 minutes (Figure 3). Asoka gave two specific bands, a yellow band at 0.95 Rf and a shining violet band at 0.25 R_f which were absent in polyalthia. Both developed a common blue coloured band at 0.50 R_f. Asoka bark samples were collected from 22 different markets of different parts of Kerala and analyzed for its authenticity using this new TLC screening method developed. Fifteen market samples tested gave characteristic 0.95 yellow band as well as 0.25 violet band confirming the genuineness of market sample as asoka whereas 7 samples could not give the characteristic spots proving the adulterant nature. It proved that these specific spots present in asoka and polyalthia can differentiate the two samples on TLC analysis (Figure 4).

Preliminary fingerprinting experiments by TLC conducted in our laboratory revealed a lot of similarities between asoka and polyalthia rather than differences. Then the solvent systems were modified and we could find suitable mobile systems effective in differentiating between the two plants. Results proved that the technique was specific and reproducible. The two different chromatographic profiles developed by asoka and polyalthia were very clear and effectively distinguished asoka from the adulterant polyalthia. Hence, this can be used as a simple tool for authentication of genuine asoka bark samples. R_f 0.95(yellow) and R_f 0.25 (violet)bands under UV-365 nm were specific to asoka only, absent for polyalthia (Figure. 3). The specific bands present in asoka which were absent in polyalthia can be of the compound/ compounds which can be taken as markers for distinguishing true asoka samples from spurious samples like polyalthia. Out of 22, asoka bark market samples analysed, fifteen were genuine asoka and seven were spurious samples. Survey of literature revealed that no quick, and reliable ,at the same time low cost methods are reported so far to assess the quality of raw drug asoka and to check the adulteration, if any , in *Saraca asoca*. A comparative anatomical analysis of the transverse sections of the bark is reported by Remashree *et al.* in 2004 to differentiate *Saraca asoca* which demands expertise. Recently Khatoon *et al.* (2009) has developed a HPTLC (High Pressure Thin Layer Chromato-graphy) method , of course a costly method, for the quality evaluation of asoka bark. HPTLC equipment is required for conducting the test. But the TLC method presently developed

in this study is rather cheap as it requires no costly equipments and chemicals and is quick, completing within 30-40 minutes. It demands no special expertise also.

Hence, this tool can be effectively employed for the quality evaluation of raw drug of asoka bark. This study demonstrates the potential of TLC technique as a rapid, easy and cheap fingerprinting tool for the authentication and quality assessment of the important raw herbal drug asoka bark and also to screen the commercial samples for adulteration. This study also confirms wide adulteration in Kerala market samples.

Figure 1: *Saraca asoca* (Roxb.) *de Wilde*

Figure 2: *Polyalthia longifolia* (Sonn.) *Thwaites*

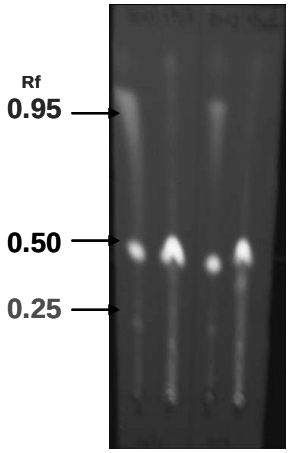
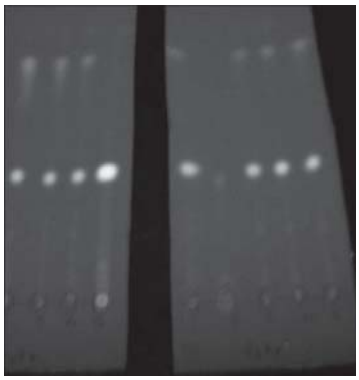
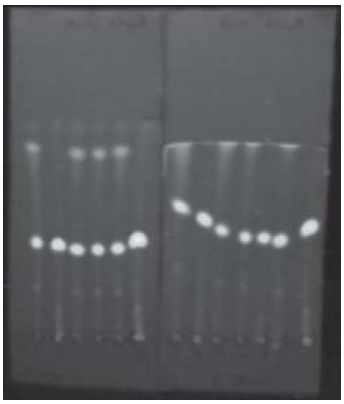


Figure 3. Petroleum ether extract Mobile phase-Benzene: Ethyl acetate (9.7:0.3) sprayed with antimony trichloride reagent and heated at 90 ° C for 5 minutes A_1,A_2 - Asoka, P_1,P_2 - Polyalthia

Figure 4. TLC fingerprint of different 22 asoka bark market samples . 15 market samples show the characteristic yellow band as well as the violet band specific for genuine asoka bark . Other 7 samples are non asoka.





Petroleum ether extract
Mobile phase - Benzene: Ethyl acetate (9.7:0.3)
Sprayed with Antimony trichloride Reagent and heated at 90° C for 5 minutes

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