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Pharmacognostic evaluation of ayurvedic drug sariva and its commonly known adulterant and substitutes

Ankita Misra[#], Mridul Kant Chaudhary[#] & Sharad Srivastava[*]
Pharmacognosy Division, CSIR-National Botanical Research institute, Lucknow 226 001, U.P., India
[*]E-mail: sharad_ks2003@gmail.com

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Sariva is an important Ayurvedic drug designated to *Hemidesmus indicus* L. in Ayurvedic Pharmacopoeia of India. Roots of this plant are used either singly or as an ingredient in various Ayurvedic formulations and claimed to be useful in cough, fever, inflammation, gout, menorrhagia, blood purification, kidney and urinary disorders. Due to its high commercial demand, locals in different parts of India use few other species, namely, *Ichnocarpus frutescens* (L.) W. T. Aiton, *Decalepis salicifolia* Wight & Arn and, *Cryptolepis buchanani* Roem. & Schult. in name of Sariva. We carried out a comparative pharmacognostic evaluation of all the four species sold in name of Sariva to check the possible adulterants or substitute to the genuine one *i.e., Hemidesmus indicus.* These developed diagnostic (pharmacognostical) markers based on the physicochemical parameters and vanillin content will be useful in quality check for the bulk procurement by various herbal drug industries.

Keywords: *Cryptolepis buchanani, Decalepis salicifolia, Hemidesmus indicus, Ichnocarpus frutescens,* Vanillin

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Sariva is an important Ayurvedic drug officially known to medicinal species *Hemidesmus indicus* L. designated in "The Ayurvedic Pharmacopoeia of India". Roots of this plant are used either singly or as an ingredient in various Ayurvedic formulations like *Sarivadyasava, Sarivadyavaleha, Sarivadi vati, Sarivadi louha, Sarivadi Kasaya and Pinda taila*[1] etc. and claimed to be useful in cough, fever, inflammation, gout, menorrhagia, blood purification, kidney and urinary disorders[2-5]. Due to its high commercial demand, locals inhabiting in different parts of India use various plant species under Sariva[6], which will lead to adulteration and substitution of the authentic species. The Indian herbal drug industry has a sustained issue for procurement of good quality raw material, due to various intension or unintentional practices. This will lead to addition of inferior quality raw material, and ultimately the efficacy of product is compromised. In the present context, on market survey, we found that there are few other species, namely, *Ichnocarpus frutescens* (L.) W. T. Aiton, *Decalepis salicifolia* Wight & Arn and, *Cryptolepis buchanani* Roem. & Schult., which are available in name of *Sariva*. In southern part of India, *Decalepis* sp. is being used in the name of Sariva[7], in Northern and Central India *Hemidesmus indicus* is being used, however, *Cryptolepis buchanani* is used in Western and Deccan zone of India. Various reports are available on the phytochemical constituents on all these four species[2,8-13]. However, in all the four species, presence of vanillin and its isomer 2-Hydroxy-4-methoxybenzaldehyde is common[14].

Thus, a comparative pharmacognostic evaluation of all the four species sold under the name of sariva was carried out to check the possible adulterants or substitute to the genuine species *i.e., Hemidesmus indicus.* These developed pharmacognostic markers based on the physicochemical parameters and vanillin content will be useful to screen and in quality check for the bulk procurement of raw material/finished formulations by various herbal drug industries.

Materials and Methods

Reagents

Vanillin (Vanillin, >97%) was procured from Sigma-Aldrich (St. Louis, MO, USA) and TLC plates (aluminum precoated with 0.2 mm layer) silica gel

*Corresponding author
[#]both authors contributed equally

$60F_{254}$ were procured from Merck (Germany). All the other chemical and reagents were of HPLC grade (SD fines, India).

Plant material

The roots of *Hemidesmus indicus* L.*, Ichnocarpus frutescens* (L.) W. T. Aiton*, Decalepis salicifolia* Wight & Arnand, *Cryptolepis buchanani* Roem. & Schult. were collected from different locations of India. The brief passport datasheet was prepared to record the prevailing GPS conditions of the species inhabiting location. The species were authenticated, herbarium specimen was deposited and voucher collection number was assigned to each species (Table 1).

Pharmacognostical studies

The various physico-chemical parameters *i.e.,* foreign organic matter, Loss on drying (moisture content), ash values *viz.,* total ash, acid insoluble ash, water soluble ash and extractive values *i.e.,* hexane soluble, alcohol soluble and water soluble) were analyzed as per Ayurvedic pharmacopeia of India. The percentage of various primary and secondary metabolites like sugar[15], starch[15], phenolics[16], flavonoid[17], tannin[18] and saponin[19] were estimated in roots as per the standard protocols.

Quantification of metabolites through high performance thin layer chromatography

Preparation of plant extract

The dried roots were chopped and grinded into a coarse powder (40 mesh sieve), about 5 g of defatted (with petroleum ether) materials was subjected to cold maceration with methanol (25 mL). The mixture was allowed for extraction till 18 h with intermittent shaking, followed by standing period of 6 h. It was then filtered through Whatmann no. 1 filter paper and residue was resuspended in fresh solvent. The extraction process was repeated three times and filtrate (pooled) was concentrated in Rota-vapor under reduced condition of temperature (45°C±2) and pressure (40 mbar) to dry residue. The extract was then lyophilized and weight.

Instrumentation and chromatographic conditions

The HPTLC quantification of vanillin was carried out on Camag HPTLC automated system. A CAMAG ATS 4 (automatic TLC sample applicator) was used to apply the aliquots of standard and extract, as a band on HPTLC plate. The plates were then developed in CAMAG ADC-2, an automated chamber with humidity control. The slit dimensions were 5 x 0.35 mm and speed of scanning was 100 mm/s. CAMAG TLC Scanner was used for scanning of band and data evaluation was done through vision CATS software (version 3.2.1, Switzerland) in absorbance–reflectance mode. The quantification was carried out on 20x10 cm TLC aluminum plates, precoated with silica gel $60F_{254}$. Tracks of sample and standard were applied as 8 mm band with automated TLC sampler under nitrogen flow. The chromatogram was allowed to develop in linear ascending manner under automated developing chamber. The saturation and solvent system were optimized at standard condition of temperature (25°C±2) and humidity (55%±2) for the better resolution of chromatogram. The plate was allowed to develop at a height of 8 cm from the point of band application and the run time was standardized. After the development of chromatogram, plate was air dried and densitometric scanning was performed at absorption maxima of standard marker in absorbance-reflectance mode. The vanillin was quantified on percentage dry wt. basis of powdered sample[20,21].

***In vitro* anti radical assay**

The radical scavenging potential of four species was carried out through DPPH radical scavenging assay[22] and ORAC assay[23].

Statistical analysis

The data was represented as mean ± S.D (n=3), for each observation. The data was subjected to one-way ANOVA to test the level of significance (XLSTAT, 2010, Microsoft Corporation, USA) at 5%.

Table 1 — Brief passport data sheet of the four sariva species

S. No.	Species	Field voucher number	Place/location	GPS coordinates			Soil type	Extractive value (%)
				Height (Meter)	Latitude	Longitude		
1.	*Hemidesmus indicus*	265411	Secunderabad, Telangana	593	17°32’13”	78°34’16”	Loamy & clayey	24.86
2.	*Ichnocarpus frutescens*	265412	Lucknow, Uttar Pradesh	111	26°47’58”	81°00’28”	Loamy sandy	21.3
3.	*Decalepsis saclisifolia*	265413	Mysore, Karnataka	2058	13°41’22”	75°14’41”	Clayey	19.6
4.	*Cryptolepis buchanani*	265414	Chitrakoot, Madhya Pradesh	460	25°09’22”	80°51’47”	Sandy	16.05

Results and Discussion

The samples were collected after thorough identification and therefore the foreign organic matter was found nil. The various physico-chemical parameters reveal that the values of three species are at par with *Hemidesmus indicus.* Moisture content was found to be highest in *Ichnocarpus*, followed by *Hemidesmus*, *Cryptolepis* and *Decalepis* sp. respectively. The total, acid insoluble and water-soluble ash within the species varies from 3.75-2.9%, 0.2-1%, and 1.5-2.6%, respectively. The water-soluble extractive value of each species was found higher than its alcohol soluble and hexane soluble extractive value. The extractive values are indicator of the for determination/identification of exhaustive or adulterated raw material and also give information regarding the class of metabolites present in species with respect to the polarity of solvent used. In this context, the Sariva species were found rich in polar metabolites having affinity towards water and resulting in high water-soluble extractive value (Fig. 1a). The quantitative estimation of phytochemicals in sariva species suggested that saponin content was significantly higher than other estimated metabolites. This is supported with above result(s), saponin(s) being water soluble metabolites might have extracted in water and resulting in high water-soluble extractive value. This is followed by starch, sugar, phenolics, and flavonoid content. The tannin content was found lowest in targeted species which is justified as they are abundantly available in bark (Fig. 1b). The HPTLC quantification of vanillin was carried out in a binary solvent system of normal hexane and ethyl acetate (8:2 v/v). The working solution of standard and sample of concentration 1 mg/mL and 10 mg/mL was applied as band on the plate and chromatogram was allowed to developed in twin trough chamber, pre-saturated 20 min before the development under controlled condition of temperature and relative humidity. The vanillin was separated from various other unknown markers present in plant extract and was identified R_f 0.192±0.2. The spectral scanning of standard and sample was performed from 200 to 800 nm in absorbance-reflectance mode. Densitometric quantification of vanillin at absorption maximum of 310 nm reveals that the content varies from 0.0042 to 0.099 mg/mL. The metabolite content in targeted species is statistically insignificant ($p<0.05$), the maximum content was in *Hemidesmus indicus* (0.099 mg/mL) followed by *Ichnocarpus frutescens* (0.0679 mg/mL) and *Cryptolepis buchanani* (0.0653 mg/mL). Least content of vanillin *i.e.,* 0.0092 mg/mL was found in *Decalepis salicifolia* (Fig. 2). Vanillin and its analogue 2-hydroxy-4-methoxybenzaldehyde is an aromatic phenolic, responsible for the sweet characteristic fragrance of *H. indicus* and is worldwide one of the most demanding flavoring agents in food, cosmetics and pharmaceutical industries. Besides, it also exhibits several pharmacological activities like anti-hyperlipidemic, anti-diabetic, Hepatoprotective, anti-diarrheal, anti-aflatoxigenic etc.[24]. The major bioactive metabolite *i.e.,* vanillin in Indian sarsaparilla is phenolic aldehyde in nature and hence, radical scavenging potential of four species were also evaluated. DPPH radical scavenging assay is frequently used to access the ability of test extract to quench the unstable radicals and the underlying mechanism is well established. The percentage inhibition was tested at five variable dilutions ranging from 10 to 50 mg/mL.

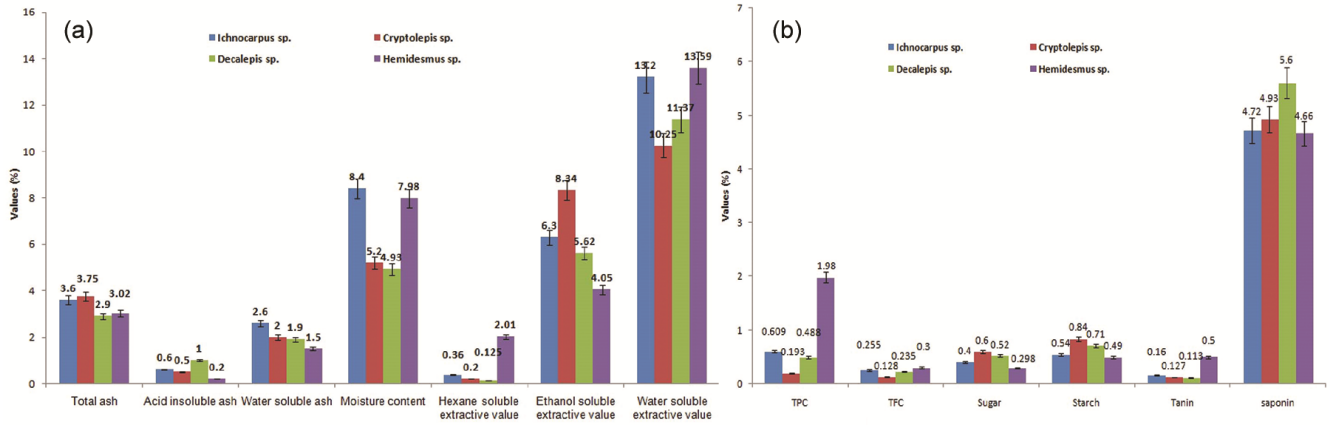

Fig. 1 — (a) Various physico-chemical parameters of four *Sariva* species, (b) Various phytochemical parameters of four *Sariva* species (TPC: total phenolic content, TFC: total flavonoid content)

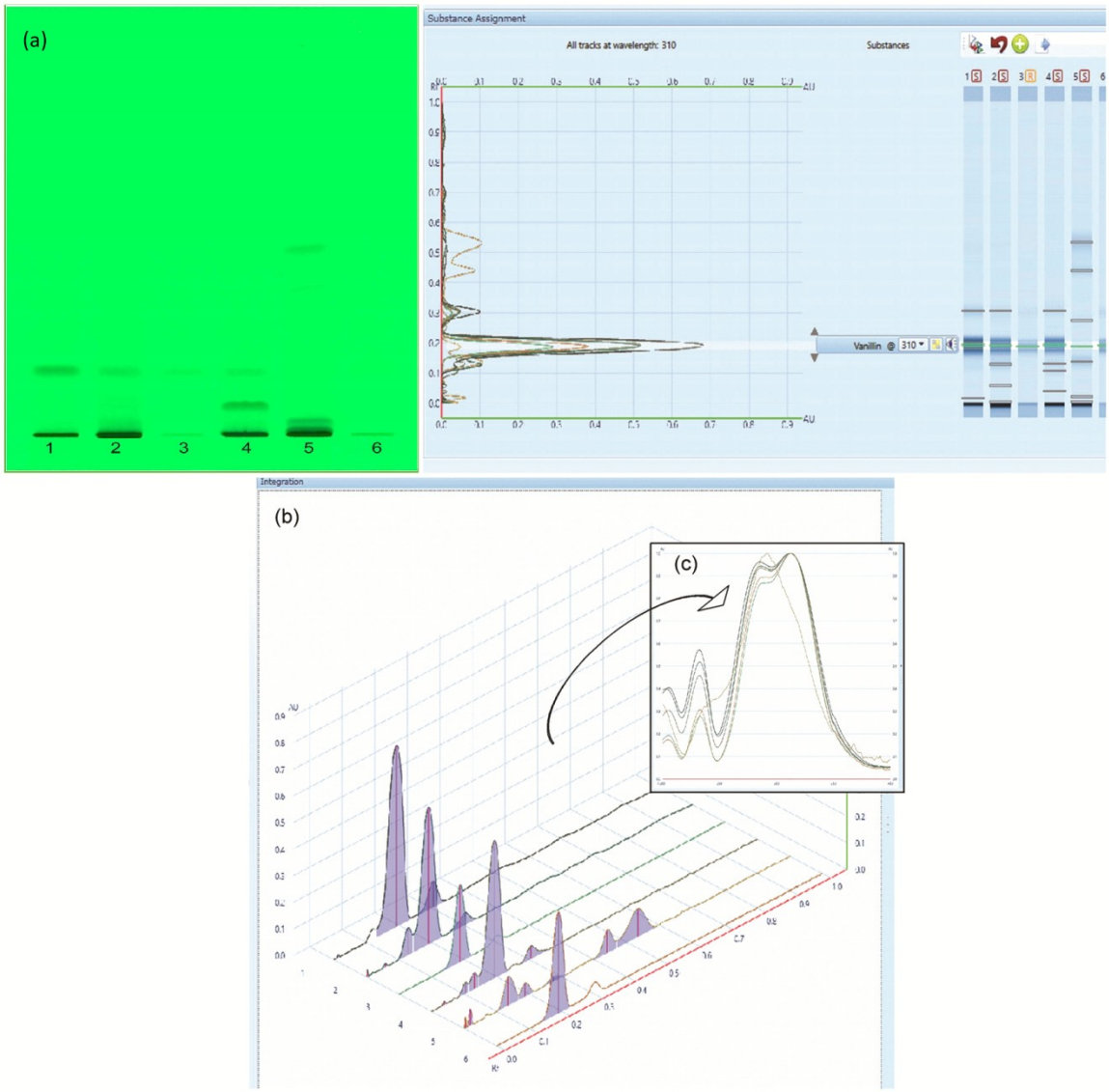

Fig. 2 — HPTLC quantification of Vanillin in four sariva species. (a) HPTLC chromatogram of standard and samples at UV_{254} nm. (b) Overlay spectra of standard and samples at absorption maxima of 310 nm. (c) Spectral scanning of standard and sample in UV–visible range of 200 to 800 nm. (1: *Hemidesmus indicus*, 2: *Ichnocarpus frutescence*, 3: Standard vanillin, 4: *Crytolepis buchnani*, 5: *Decalepsis salicifolia*

IC_{50} of *Hemidesmus* was found lowest *i.e.,* 20.02 mg/mL and thus exhibits the most promising activity, percentage inhibition ranges from 20.24 to 94.68%. Further, *Ichnocarpus frutescens* showed 90.13% inhibition and IC_{50} value was at 27.33 mg/mL. whereas, in *Decalepis salicifolia* and *Cryptolepis buchanani*, inhibition (%) ranges from 13.10 to 67.32% and 10.91 to 44.98% respectively, exhibiting the IC_{50} at 36.35 and 56.82%. The standard gallic acid under the same working protocol showed 84.36% inhibition and IC_{50} value at 14.37 mg/mL. The antioxidant potential of plant extract to scavenge peroxyl radicals initiated by spontaneous decomposition of 2'-azobis (2-amidino-propane) dihydrochloride (AAPH) was evaluated in terms of ascorbic acid equivalent (ASE). The ORAC value exhibited by test extracts (methanol) showed good antioxidant activity among the samples (Fig. 3). The highest ORAC value was found in *Hemidesmus indicus* (4149677 µmol ASE/g) followed by *Decalepis salicifolia* (1708139 µmol ASE/g), *Cryptolepis buchnani* (1030420 µmol ASE/g) and *Ichnocarpus frutescens* (881793.1 µmol ASE/g). From the results it is concluded that the species are rich source of phenolic compounds and therefore possesses high radical scavenging potential.

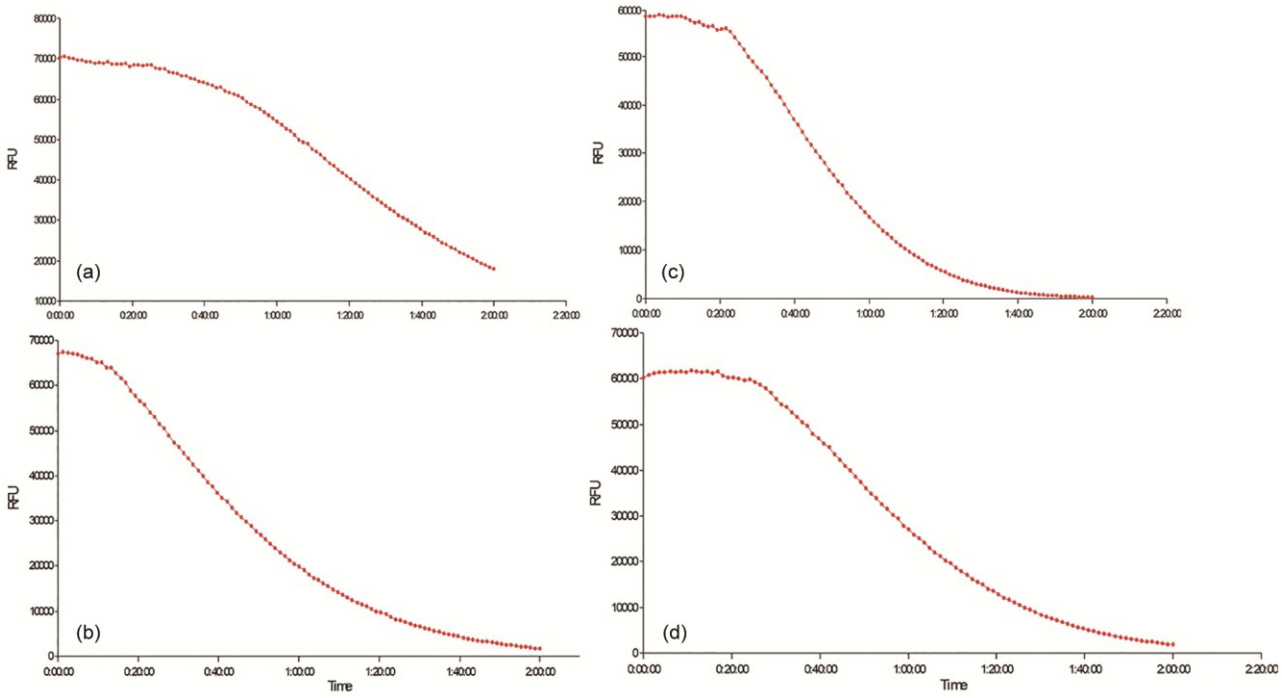

Fig. 3 — Fluorescein decay curve of (a) *Hemidesmus indicus*, (b) *Ichnocarpus frutescens*, (c) *Cryptolepis buchnani*, (d) *Decalepsis salicifolia*

Conclusion

A comparative evaluation of all the four species revealed (in terms of its physicochemical and phytochemical parameters) that *Ichnocarpus frutescens, Decalepis salicifolia* and *Cryptolepis buchanani* are comparable to *Hemidesmus indicus* which is the official drug as per Ayurvedic Pharmacopoeia of India. The Vanillin content was maximum in *Hemidesmus indicus*, however, vanillin is also present in other three species but in a little lesser quantity than *Hemidesmus indicus*. The study will be useful in quality check of Sariva used by various herbal drug industries through the developed diagnostic (pharmacognostic) markers. In mind that substitutes and adulterants of shares similar property with authentic species, but it is equally important to note that the effectiveness of an Ayurvedic drug relies on its unique combination of rasa, guna, veerya, and vipakas - also known as rasapanchakas. These properties may differ in substitute drugs, making it necessary to conduct clinical studies to evaluate their efficacy.

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Conflict of Interest

None

Author Contributions

AM: data analysis, interpretation and writing original draft; MKC: data analysis and writing original draft; SS: Supervision, manuscript draft review and editing.

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