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An Exploratory Study on *Asokarishtam* an Ayurvedic Formulation with Leaves of *Saraca Asoka*

Sruthi CV[1*], Vendamirtham S[1], Asha K Maniyan[1], Neeraj PT[1], Sunitha V[1], Sindhu A[1]

1.Research and Development division, The Arya Vaidya Pharmacy (Coimbatore) Limited, Kanjikode,Palakkad,Kerala-678621

ABSTRACT

Asokarishtam, prepared using the bark of *Asoka* (*Saraca asoka (Roxb.) de.wilde*) is widely used in the management of uterine diseases. The scarcity of genuine *Asoka* bark is a problem facing by the ayurvedic industries and alternative to it without affecting the efficacy has become an urgent requirement. The present study explores whether leaf of *Saraca asoka (Roxb.) de.wilde* is an appropriate alternate for the bark of *Saraca asoka (Roxb.) de.wilde.* The physicochemical and phytochemical analysis were performed using standard procedures. The total phenolic content was determined using Folin-ciocalteu's method, tannin content by Folin-Denis Method, flavonoids by Aluminium chloride colorimetric method and radical scavenging activity by 2'2-diphenyl-1-picryl hydrazine method. Thin layer chromatographic profiling was also performed on precoated silica gel aluminium plate. The physicochemical and phytochemical analysis of Asokarishtam with leaf and Asokarishtam with bark do not show any significant variation. The total phenolic content (6.47 %, 7.33 %), tannin content (6.02 %, 5.79 %), flavonoid content (0.775 %, 0.786 %) and radical scavenging activity (20 µg/ml, 18 µg/ml) of both Asokarishtam with leaf and bark are comparable. Thin layer chromatographic profiling shows similar Rf in Asokarishtam with leaf and bark. From this study it is concluded that there is a probability of substituting leaf of *Saraca asoka (Roxb.) de.wilde* for its bark. Activity evaluations including In-Vivo and Toxicological studies are proposed before confirming the usage of leaves for the bark in the preparation of *Asokarishtam.*

Keywords: *Saraca asoka (Roxb.) de.wilde*, *Asokarishtam*, bark, leaf

*Corresponding Author Email: avpfact.qc@gmail.com

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INTRODUCTION

Asokarishtam, an aqua alcoholic preparation using the bark of *Asoka Saraca asoka (Roxb.) de.wilde* is mentioned in the text of *Bhaishajyaratnavali* in the context of uterine diseases [1]. Bark of *Asoka*, the major constituent of the *Arishtam* is highly astringent, uterine sedative and effective in conditions like menorrhagia, dysmenorrhoea [2].

Asoka bark being the major component of the *Arishtam* is required in a sizeable quantity for the production of a standard batch of *Asokarishtam*. The scarcity of genuine *Asoka* bark [3] is a stumbling block to ayurvedic industries in the manufacturing of *Asokarishtam*. The yield of the bark from a tree is very less and duration of the regeneration of the bark is more. Unscientific harvesting of the bark results in the damage of the trees. Moreover the natural occurrence of *Saraca asoka (Roxb.) de.wilde*is also drastically reduced due to various other reasons. All these reasons contribute to the scarcity of *Saraca asoka (Roxb.) de.wilde* and its non availability in the Ayurvedic industries. An alternative to *Asoka* bark which could be used in the preparation without affecting the efficacy of the product and could be sustainably harvested has become an urgent requirement.

The present study explores whether leaf of *Saraca asoka (Roxb.) de.wilde* is an appropriate alternate for the bark of *Saraca asoka (Roxb.) de.wilde*

MATERIALS AND METHODS

Sample collection

Leaves and bark of *Saraca asoka (Roxb.) de.wilde* were collected from the medicinal plant cultivation farm of The Arya Vaidya Pharmacy (Coimbatore) Limited, Kanjikode, Palakkad, Kerala. Sample were taken from branch and main trunk of the tree from a height of 5 ft (GBH) of the trunk and the thickness of the bark is approximately 5mm-1 cm

The samples of both leaves and bark of *Saraca asoka (Roxb.) de.wilde* were authenticated by Prof.V.Vasudevan Nair (Retd. Professor. Govt. Victoria College, Palakkad) Consultant Taxonomist, The Arya Vaidya Pharmacy (Coimbatore) Limited

Method of preparation

The samples for the study is prepared from both the leaves and bark of *Saraca asoka (Roxb.) de.wilde* Water extract of both leaves and bark are prepared separately by classical procedure of *kashaya* preparation[1] The sweetener jaggery is dissolved in the above prepared water extract and filtered. On cooling it is transferred to a stainless steel vessel meant for fermentation and after that fermenting aid (*Dhathaki pushpa*) and Powder drugs (*prakshepa choorna*) are added and

kept for 30 days to complete fermentation. It is then filtered, kept for sedimentation and the supernatant solution thus obtained is used for further study.

Preliminary physicochemical and phytochemical screening

The physicochemical parameters like specific gravity, pH, total solids, acidity and alcohol percentage were determined as per the method given in The Ayurvedic pharmacopoeia of India [4]**.** Qualitative phytochemical tests for the identification of alkaloids, flavonoids, phenol, tannin, anthraquinones, saponins and terpenoids were carried out for all the formulations by the method described by Harborne 1998 and Sazada *et al.* 2009[5,6] Phytochemical screening of the formulations is performed using the following reagents and chemicals: Phenols with 5% ferric chloride, flavonoids by lead acetate reagent, tannins with 10% alcoholic ferric chloride, steroids and terpenoids with concentrated sulphuric acid and chloroform, alkaloids with Hager's, Wagner and Dragendorff's reagent, cardiac glycosides with 5% ferric chloride and glacial acetic acid and saponins by foam test.

Determination of total phenolic content

The total phenolic content was determined using Folin-ciocalteu reagent [7]. Appropriately diluted standard and samples were made up to 3.5ml with distilled water in a series of test tubes. These tubes were then treated with 0.5ml 2N Folin-ciocalteu's reagent and incubated for 3minutes at room temperature. The reaction was then neutralized by the addition of 1ml 20% sodium carbonate. The reaction mixture was then incubated at room temperature for 90 minutes after which the absorbance was read at 760 nm (Shimadzu UV Vis spectrophotometer, 1800) and the percentage phenolic content is calculated from the graph

Quantitative determination of tannin:

The tannin content in Arishtam is estimated by Folin-Denis Method[8]. Tannin like compounds reduces phosphotungstomolybdic acid in alkaline solution to produce a highly coloured blue solution, the intensity of which is proportional to the amount of tannins present. The intensity is measured using UV-Vis spectrophotometer (Shimadzu UV Vis spectrophotometer, 1800) at700nm.The standard tannic acid is prepared by dissolving 100 mg of tannic acid in 100 ml of distilled water. To100µl of appropriately diluted arishtam 0.5ml Folin-Dennis reagent 1ml Sodium carbonate solution are added and made upto 3.5 ml with distilled water. It was incubated for 30 minutes. The absorbance is read using Shimadzu UV-Vis spectrophotometer (1800). The percentage of tannin is calculated.

Total flavonoids determination

Aluminum chloride colorimetric method was used for flavonoids determination[9] Each sample

were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 3.3 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a UV/Visible spectrophotometer (Shimadzu UV Vis spectrophotometer, 1800) the calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 g in methanol.

TLC fingerprint profiling

Thin layer chromatography was performed on a precoated silica gel aluminium plate (60 F_{254} plates, Merck) for chromatographic separation of various extracts of the *arishtams*. Briefly, 25ml of each *Arishtam* were mixed with 10g of silica and dried. This was then subjected to successive solvent extraction in soxhlet apparatus using solvents in increasing order of polarity like hexane, ethyl acetate, water and methanol. The extracts were then concentrated and used for spotting on TLC plate. Solvent system used were Petroleum ether: Acetone (9:2), Benzene: Chloroform: Methanol (5:5:2), Butanol: Acetic acid: Water (8:2:1), Ethyl acetate: acetone: Methanol (4:3:1) respectively. The spraying reagent used was anisaldehyde sulphuric acid reagent.

DPPH radical scavenging activity

The antioxidant activities of the Ayurvedic formulations were estimated by DPPH (2'2-diphenyl-1-picryl hydrazine) radical scavenging activity[10]. Briefly, to various concentrations of the sample, methanolic solution containing DPPH radicals (0.1mM) was added and shaken vigorously. The reaction mixture was then left to stand for 30minutes in dark. After the incubation period, the absorbance was measured at 517nm against the corresponding test blanks. The percentage inhibition of DPPH free radical was calculated using the formula, calculated using the formula,

% Inhibition = ((Control-sample)/Control) X 100

The sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph of radical scavenging activity percentage against sample concentration. Gallic acid was used as standard.

RESULTS AND DISCUSSION

The physicochemical parameters of kashayam prepared by leaves and bark do not show any significant variation. The results is summarized in table1 . The physicochemical parameters of AA-L and AA-B do not show any significant variation except that AA-L shows an increase in alcohol%. The results are summarized in table 2.

Table 1: Physicochemical analysis of kashayam

Sl No	Parameters	Leaf	Bark
1	Specific gravity	1.000	1.000
2	% Total solids	1.592	1.613
3	pH	5.31	5.44

Table 2: Result of physicochemical analysis of arishtam

Sl No	Parameters	Result (AA-L)	Result (AA-B)
1	Alcohol	12.95	9.77
2	Acidity	0.56	0.57
3	pH	3.73	3.22
4	Specific gravity	1.010	1.010
5	% Total solids	8.036	8.164

The preliminary phytochemical analysis revealed the presence of carbohydrates, phenols, tannins, terpenoids, glycosides and saponins in both AA-L and AA-B (table 3). The phytochemical analysis of leaves and bark of *Saraca asoka (Roxb.) de.wilde* reveals the presence of flavonoids, tannins, terpenoids, sterols and saponins [11, 12, 13] The thin layer chromatographic profiling of the four extracts of both AAL and AAB are comparable (figure 1-4). The Rf values are calculated and tabulated in table 4.

Table 3: Result of Preliminary phytochemical screening.

Secondary Metabolites tested	Test performed	AA-B	AA-L
Phenol	Phosphomolybdic acid test	Present	Present
Flavonoid	Lead acetate test	Absent	Absent
Tannins	Braemer's test	Present	Present
Terpenes	Liebermann-burchardt test	Present	Present
Steroids	Liebermann-burchardt test	Absent	Absent
Alkaloid	Dragendorff's test, Hager's test, Wagner's test	Absent	Absent
Glycosides	Legal's test	Present	Present
Saponins	Foam test	Present	Present
Anthraquinone	Borntrager's test	Present	Absent

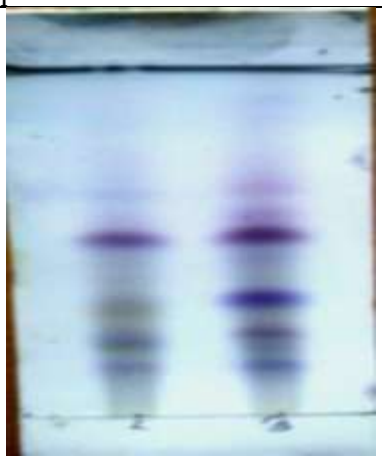

Figure 1 & 2: Hexane extract of AA-L,AA-B Ethyl acetate extract of AA-L,AA-B

Figure 3: Water extract of AA-L,AA-B

Lane 1 AA-L, Lane 2 AA-B

Figure 4 Methanol extract of AA-L,AA-B

Table 4: Thin layer chromatographic Rf Values

	Hexane extract		**Ethyl acetate extract**		**Water extract**		**Methanol extract**	
	AA-L	AA-B	AA-L	AA-B	AA-L	AA-B	AA-L	AA-B
Rf* values	0.14 0.22 0.34 0.49 0.57 0.65	0.14 0.22 0.34 0.51 0.57 0.65	0.08 0.16 0.62 0.72 0.83	0.08 0.16 0.59 0.71 0.83	0.16 0.40	0.16 0.40	0.08 0.41 0.72 0.91	0.08 0.41 0.72 0.91

***Retention factor**

Phenolic compounds, including flavonoids have been reported to have multiple biological effects and have potential health benefiting properties [14]. The estimation of these secondary metabolites are performed and the results are shown in table 5. The results indicate that the total phenolic content, Flavanoid% and tannin % in both AA-L and AA-B are comparable. The total phenolic content of AA-L and AA-B is 6.47% and 7.33%, flavonoid content 0.775% and 0.786%, and tannin content 6.02% and 5.79% respectively. Phytochemical analysis of *Saraca asoka (Roxb.) de.wilde* bark showed a maximum total phenolic content/ concentration of 7.25 $\pm$ 0.94% and Flavonoid content (0.23 $\pm$ 0.04%) tannin (6.55%) [15, 16, 17].

Table 5: Result of quantitative estimation of secondary metabolites

Sl No:	**Secondary metabolites**	**Observed results**	
		AA-L	**AA-B**
1.	Total phenolic content (%)	6.47	7.33
2.	Total flavonoids (%)	0.775	0.786
3.	Tannin (%)	6.02	5.79

Asokarishtam is clinically used in *Asrigdharam*, *Ruja* and *Sotha* which can be related to conditions like dysmenorrhoea, menorrhagia and pelvic inflammatory disorders. It is studied that

oxidative stress contributes to the etiology of dysmenorrhoea, menorrhagia and pelvic inflammatory disorders [18]. The secondary metabolites of herbs in the plant based products help to relieve the oxidative stress induced by the free radicals .The unique structure of the secondary metabolites like phenolic compounds, contribute to their antioxidant property [19]. The Antioxidant properties of AA-L and AA-B are studied and their results are found to be comparable. Thus AA-L also addresses one major property which AA-B possesses and contributes to most of its attributed properties (Table 6, figure 5).

Table 6: In vitro antioxidant activity

Samples	*IC $_{50}$
Asokarishtam with Leaf	20 µg/ml
Asokarishtam with Bark	18 µg/ml

*Inhibitory concentration for 50% inhibition

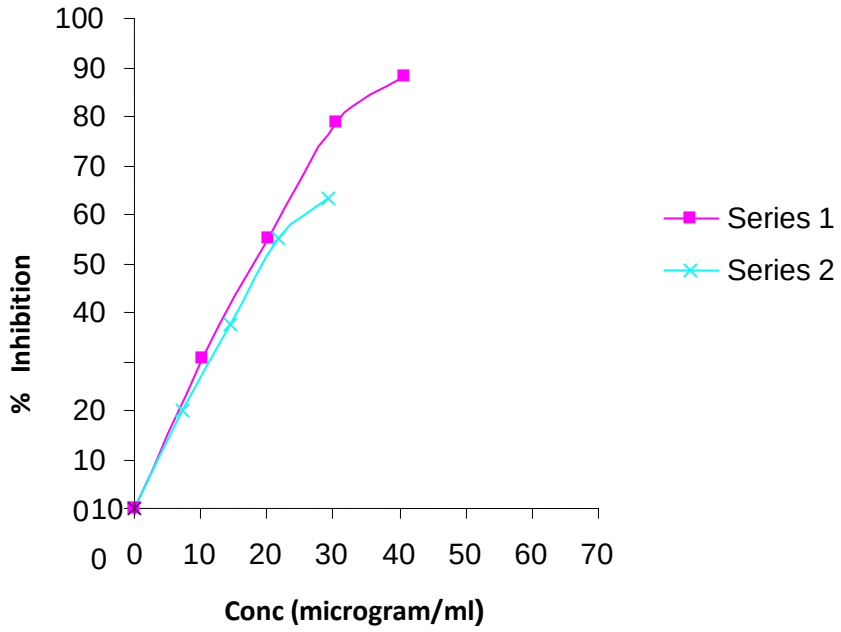

Figure 5: In-vitro antioxidant activity of AA-L , AA-B Series 1: AA-B; Series 2: AA-L

CONCLUSION

This exploratory study on the basis of preliminary phytochemical analysis, quantitative analysis and antioxidant property provides a probability of substituting leaf of S.asoka for its bark. Activity evaluations including In-Vivo and Toxicological studies are proposed before confirming the usage of leaves for the bark in the preparation of *Asokarishtam.*

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