Research Article.

International Research Journal of Ayurveda & Yoga

An International Peer Reviewed Journal for Ayurveda & Yoga

A Comparative Analysis of *Churna* and *Bhavita Churna* of Stem Bark of *Asoka – Saraca asoca* (Roxb.) de Wilde through High Performance Thin Layer Chromatography

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VOLUME 4 ISSUE 9

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Article received on 24th August 2021 Article Accepted 26th Sept. 2021 Article published 30 Sept. 2021

ABSTRACT: -

High performance thin layer chromatography is a powerful analytical technique used in the process of drug discovery. The fingerprint profile developed for each drug using this technique will be unique which makes it useful in establishing identity of a drug. The quality of different drug samples can be compared qualitatively and quantitatively through this technique. *Asoka – Saraca asoca* (Roxb.) de Wilde is a plant that has immense therapeutic potential. It is a drug which is significantly related to disorders of women. The stem barks are widely utilized for reproductive disorders like heavy and prolonged menstrual bleeding. The stem bark possesses several phytoconstituents including flavonoids, polyphenolics and sterols which accounts for its multiple beneficial action in therapeutics. The *churna* (powder) and *bhavita churna* (processed powder) of stem bark of *Asoka – Saraca asoca* (Roxb.) de Wilde was subjected to high performance thin layer chromatography and comparative analysis of different peaks were obtained. The number of peaks in the chromatogram of *bhavita churna* (processed powder) was more when compared to *churna* (powder) which substantiates its better potency.

Key words: High performance thin layer chromatography, *churna*, *bhavita churna*

Research Article.

How to cite this article: Kumar A.K, Shincymol V V, Ansary P Y, Oommen S. R " A comparative analysis of *churna* and *bhavita churna* of stem bark of *Asoka – Saraca asoca* (Roxb.) de Wilde through High performance thin layer chromatography, IRJAY. [Online] 2021;4(9): 1-8. Available from: http://irjay.com ;
DOI: https://doi.org/10.47223/IRJAY.2021.4901

INTRODUCTION

High Performance thin layer chromatography (HPTLC) is a powerful tool to separate phytoconstituents. It helps in authentication of crude drugs. HPTLC fingerprint of an herbal drug helps to confirm its identity. It separates compounds on the principle of adsorption. HPTLC can be used to check adulteration, monitor purity and quantify marker compounds. This separation technique is both qualitative and quantitative. The number of peaks signifies specific phytoconstituents and the area of peak implies the quantity of constituents present in the sample.[1] *Asoka*, botanically identified as *Saraca asoca* (Roxb.) de Wilde has several phytoconstituents including flavonoids like catechin, epicatechin, polyphenolics including gallic acid, ellagic acid, quercetin, lignan glycosides like lyoniside, nudiposide, sterols like stigmasterol, β-sitosterol, steroidal glycoside such as sitosterol glucoside, alkanes, esters, primary alcohols, tannin, steroids, glycosides, reducing sugars in its stem bark.[2][3] The drug has been mentioned among ten drugs of *Vedanasthapana dasemani* in *Caraka Samhita*.[4] The drugs in *dasemani* can be utilised in any of *panchavidha kashaya kalpanas – swarasa* (juice), *kalka* (bolus), *srta* (decoction), *seeta* (cold infusion) and *phanta* (hot infusion) according to the strength of patient and disease.[5] The drug when totally dried, powdered and filtered is called *churna kalpana* and is widely utilized in therapeutics. The *churna* (powder) is a modified form of *kalka kalpana* (bolus).[5] The process of repeatedly soaking *churna* (powder) in *swarasa* (juice) or *kashaya* (decoction) is known as *bhavana* (processing). It can be utilized for potentiating the *churna* (powder).[6] The powdered drug can be processed with liquid form of same drug or a different one as per the need. It can also be done for purifying the drug from its toxicity. The *churna* (powder) after subjecting to *bhavana* (processing) can be called as *bhavita churna* (processed powder). The *bhavana* procedure helps to increase the efficacy and thereby to decrease the dose of the drug. In the present study, *bhavita churna* (processed powder) of stem bark of *Asoka – Saraca asoca* (Roxb.) de Wilde has been prepared by subjecting the *churna* (powder) to seven times *bhavana* with *kashaya* (decoction) prepared out of its stem bark. The HPTLC technique was employed to compare the peaks obtained for *churna* (powder) and *bhavita churna* (processed powder) of stem bark of the drug developed using same solvents, mobile phase and stationary phase. The HPTLC profile of stem bark of the drug has been conducted in previous studies. The present study aims at comparing the peaks obtained in HPTLC chromatogram of *churna* (powder) and *bhavita churna* (processed powder) of stem bark of *Asoka – Saraca asoca* (Roxb.) de Wilde and to substantiate increased potency of *bhavita churna* (processed powder)

through *bhavana*.

MATERIALS AND METHODS

Preparation of *churna* (powder)

Fresh stem barks of *Asoka – Saraca asoca* (Roxb.) de Wilde were collected from Kodakara village of Thrissur district. The stem barks were dried under sunlight. Thereafter the properly dried stem barks were finely powdered to obtained *churna* (powder).

Preparation of *bhavita churna* (processed powder)

A sufficient quantity of whole dried stem barks was crushed and was used for the preparation of *kashaya* (decoction) for *bhavana* (processing). The crushed dried stem barks were taken in the quantity equal to that of *churna* (powder) in a mud pot. It was then added with 8 times water. The pot was kept over a firewood stove for boiling. Once started boiling, it was then reduced to $1/8^{th}$ by providing mild fire.[7] Then the *kashaya* was left undisturbed till it got completely cooled. The *churna* (powder) of the drug was taken in a clean plastic tray. It was evenly spread in a thickness of 1 cm. The prepared *kashaya* (decoction) was filtered and added slowly to the *churna* (powder) after cooling such that *kashaya* (decoction) got drained into the *churna* (powder). The powdered drug got completely submerged in *kashaya* (decoction) and a thin layer of *kashaya* (decoction) was left above the fine powder. The tray was shaken slowly for even distribution and the *churna* (powder) was mixed using a clean rod to ensure uniform mixing and was left undisturbed for whole night. The next day the soaked powder was dried in the sunlight. Once properly dried, it was again powdered to remove lumps. The *bhavana* (processing) was done in the same manner for 7 times as per reference told in *Bhaishajya Ratnavali* and the process of powdering of *churna* (powder) was done after each *bhavana* (processing).[8] After whole procedure, the properly dried *bhavita churna* (processed powder) was made into fine powder and sieved through mesh with size-120.

Figure 1. *Churna* (powder) of stem bark of *Saraca asoca* (Roxb.) de Wilde

Figure 2. *Bhavita churna* (processed powder) of stem bark of *Saraca asoca* (Roxb.) de Wilde

Procedure

The test solution for *churna* (powder) was made with 5.3843 g in 25 mL methanol and 0.4898 g was obtained as residue which was added with 1 mL methanol. For solution prepared from *bhavita churna* (processed powder), 5.2578 g was macerated in 25 mL methanol and 0.5482g was obtained as residue to which 1 mL of methanol was added. 5µl of each of the prepared solution was applied on stationary phase for HPTLC fingerprinting. Stationary phase includes HPTLC Silica gel 60 F 254, 5.0 x 10 cm aluminum sheet. Mobile phase includes Chloroform: Ethyl acetate: Methanol (8:3:1). The development of the plate was done by using CAMAG 20 x 10 cm automatic developing chamber. It was visualized under UV at 254 nm after derivatization using ferric chloride.

RESULT

HPTLC chromatogram of *churna* (powder) and *bhavita churna* (processed powder) of stem bark of *Asoka* - *Saraca asoca* (Roxb.) de Wilde were recorded. The Rf values of the separated compounds of each sample were noted at wavelength 254 nm. Each peak obtained implies specific chemical compounds that could be separated from the drug. Total 7 peaks were obtained in *churna* (powder) of stem bark of the drug. The maximum area observed in *churna* (powder) of the drug was 54688.6 AU in peak with Max Rf -0.01. Also, peaks were obtained with Max Rf 0.26 with an area 12601.6 AU, Max Rf 0.39 with area 4170.6 AU, Max Rf 0.50 with area 1449.6 AU, Max Rf 0.61 with area 3074.2 AU, Max Rf 0.75 with area 1825.3 AU, Max Rf 0.84 with area 1916.3 AU.

Table 1: Peak and area of *churna* (powder) of stem bark of *Asoka* - *Saraca asoca* (Roxb.) de Wilde at 254 nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1	-0.06	-0.01	0.18	54688.6	68.60
2	0.22	0.26	0.36	12601.6	15.81
3	0.36	0.39	0.47	4170.6	5.23
4	0.47	0.50	0.54	1449.6	1.82
5	0.54	0.61	0.69	3074.2	3.86
6	0.70	0.75	0.79	1825.3	2.29
7	0.84	0.88	0.91	1916.3	2.40

Figure 3: Overview graph of *churna* (powder) of stem bark of *Asoka* - *Saraca asoca* (Roxb.) de Wilde at 254 nm

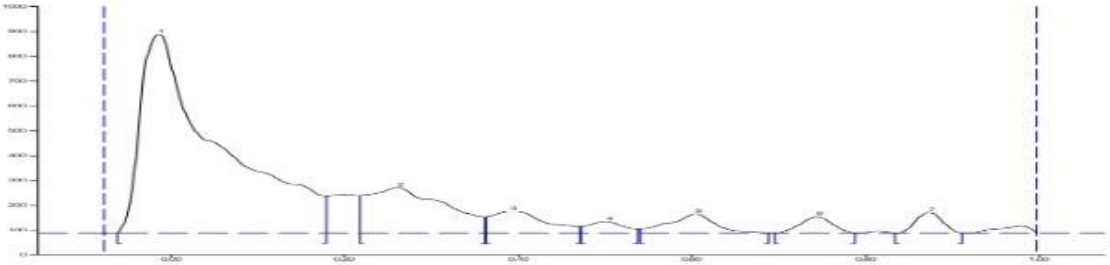
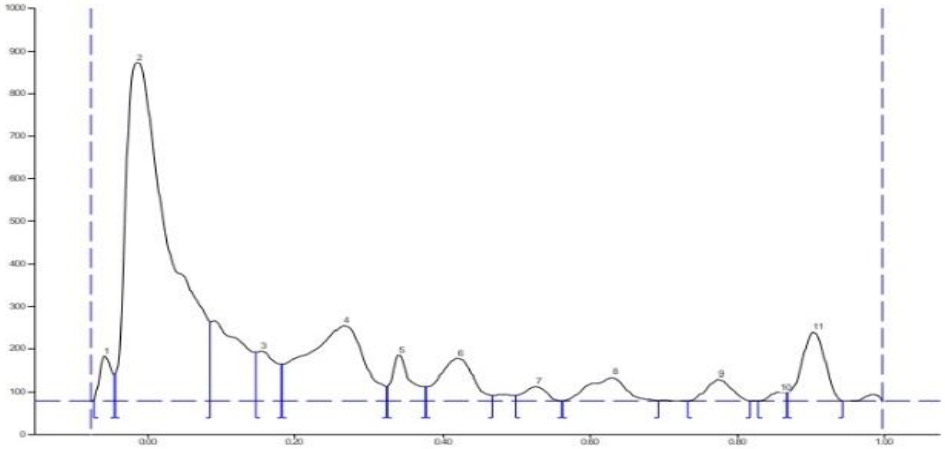


Figure 4: Overview graph of *bhavita churna* (processed powder) of stem bark of *Asoka* - *Saraca asoca* (Roxb.) de Wilde at 254 nm



In *bhavita churna* (processed powder) of the drug, 11 peaks were obtained. Peaks obtained were with Max Rf -0.06 with area 1293.6 AU. Peaks obtained were with Max Rf -0.01 having an area 35526.0 AU, with Max Rf 0.16 having an area 2439.4 AU, with Max Rf 0.27 having an area 10294.5 AU, with Max Rf 0.34 having an area 2012.8 AU, with Max Rf 0.42 having an area 3451.2 AU, with Max Rf 0.53 having an area 819.3 AU, with Max Rf 0.63 having an area of 2166.0 AU, with Max Rf 0.78 having an area of 1335.3 AU, with Max Rf 0.86 having an area of 354.2 AU, with Max Rf 0.91 having an area of 3839.8 AU. The maximum area observed in *bhavita churna* (processed powder) was 35526.0 with Max Rf -0.04

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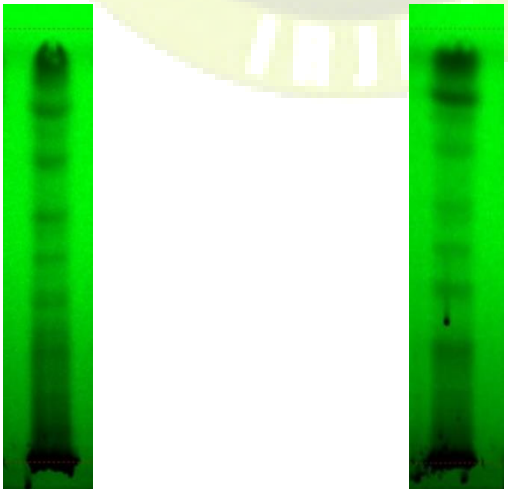
Table 2: Peak and area of *bhavita churna* (processed powder) of stem bark of *Asoka - Saraca asoca* (Roxb.) de Wilde at 254 nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1	-0.07	-0.06	-0.04	1293.6	2.04
2	-0.04	-0.01	0.09	35526.0	55.92
3	0.15	0.16	0.18	2439.4	3.84
4	0.18	0.27	0.32	10294.5	16.20
5	0.33	0.34	0.38	2012.8	3.17
6	0.38	0.42	0.47	3451.2	5.43
7	0.50	0.53	0.56	819.3	1.29
8	0.56	0.63	0.70	2166.0	3.41
9	0.74	0.78	0.82	1335.3	2.10
10	0.83	0.86	0.87	354.2	0.56
11	0.87	0.91	0.95	3839.8	6.04

Figure 5: TLC plate view of *churna* (powder) and *bhavita churna* (processed powder) of stem bark of *Asoka - Saraca asoca* (Roxb.) de Wilde at 254 nm



Churna *Bhavita churna*

DISCUSSION

HPTLC chromatogram of *churna* (powder) and *bhavita churna* (processed powder) of stem bark of *Asoka - Saraca asoca* (Roxb.) de Wilde] were recorded. The Rf values of the separated compounds of each sample were noted at wavelength 254 nm. The number of peaks obtained for *bhavita churna* (processed powder) was more when compared to *churna* (powder) of the drug indicating a greater number of phytoconstituents in *bhavita churna* (processed powder). Total number of peaks for *bhavita churna* (processed powder) was 11 in number and was 7 for *churna* (powder) of the drug. The peak intensities obtained were different for each sample, yet the presence of peaks was almost similar. Only one Rf value of -0.01 was obtained similar in both samples which indicate presence of same chemical constituent. The peak area obtained for *churna* (powder) at Max Rf value -0.01 was 54688.6 while for *bhavita churna* (processed powder), it was 35526.0. About five Max Rf values obtained in *churna* (powder) was almost similar to the peaks obtained in *bhavita churna* (processed powder) of the drug. A peak with Max Rf value 0.26 obtained in *churna* (powder) was similar to peak with Max Rf value 0.27 in *bhavita churna* (processed powder). A peak with Max Rf value 0.61 in *churna* (powder) was comparable to peak with Max Rf value 0.63 in *bhavita churna* (processed powder). Similarly, a peak with Max Rf value 0.88 in *churna* (powder) was similar to peak with Rf value 0.86 in *bhavita churna* (processed powder). Three additional peaks present in *churna* (powder) was at Max Rf value 0.39, Max Rf value 0.50, Max Rf value 0.75. HPTLC profile of *bhavita churna* (processed powder) showed some unique peaks that were not observed in *churna* (powder). These were at Max Rf value - 0.06, Max Rf value 0.16, Max Rf value 0.34, Max Rf value 0.42, Max Rf value 0.53, Max Rf value 0.78 and Max Rf value 0.91.

CONCLUSION

HPTLC profiling of *churna* (powder) and *bhavita churna* (processed powder) of *Asoka - Saraca asoca* (Roxb.) de Wilde was conducted and comparison of their peaks were discussed. *Bhavita churna* (processed powder) was found to have increased efficacy owing to the increased number of peaks in HPTLC chromatogram.

ACKNOWLEDGEMENT

I express my whole hearted gratitude to my guide Dr. Shincymol V V MD (Ay), Associate Professor, Department of Dravyaguna Vijnanam, Govt. Ayurveda College, Tripunithura for her valuable guidance and encouragement throughout the completion of this work. With deep sense of respect and love, I express my sincere gratitude to Dr. P.Y. Ansary MD (Ay), PhD, Professor and HOD, Department of Dravyaguna Vijnanam, Govt. Ayurveda College, Tripunithura, for his supervisions and suggestions to bring in perfection to this work. I am immensely grateful to Dr. Sara Moncy Oommen MD(Ay), Professor & HOD , Department of Dravyaguna Vijnanam, Govt. Ayurveda College, Tripunithura for her timely advices rendered throughout this work. I am sincerely grateful to Dr. Sethu R MD(Ay), Assistant professor and Dr. Mridula M K MD(Ay), Assistant professor, Department of Dravyaguna Vijnanam, Govt. Ayurveda College, Tripunithura their constant inspiration, valuable support, help and suggestions.

Financial Support: Nil.

Conflict of Interest: Nil

REFERENCES

1. Jamuna S, HPTLC Fingerprints of Various Secondary Metabolites in the Traditional Medicinal Herb *Hypochaeris radicata* L. Journal of Botany. 2016; Article ID 5429625, 11 pages https://doi.org/10.1155/2016/5429625

2. P. Pradhan et al. Saraca asoca (Ashoka): A Review. *Journal of Chemical and Pharmaceutical Research*. 2009; 1 (1):62-71.

3. Borokar A. A, Plant Profile, Phytochemistry and Pharmacology of Ashoka (Saraca Asoca (Roxb.) De. Wilde) – 4. A Comprehensive Review. *International Journal of Ayurvedic and Herbal medicine*.2017; 7(2): 2524-2541.

5. Kavyatirtha N R A, *Caraka Samhita*. New Delhi: Chaukhamba Publications; 2016, pp.34.

6. Kavyatirtha N R A ,*Caraka Samhita*. Trikamji J New Delhi: Chaukhamba Publications; 2016, pp.31.

7. Kavyatirtha N R A *Caraka Samhita*. Trikamji J New Delhi: Chaukhamba Publications; 2016, pp.672.

8. Govinda D, *Bhaishajya rathnavali*. Vol 1. Varanasi: Chaukambha Sanskrit Sansthan; 2008, pp. 89-90.