TLC AND HPLC Fingerprint development of *Aegle marmelos* Corr. And its polyherbal marketed formulations

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Abstract

Chromatographic fingerprint analysis of herbal drugs represents a comprehensive qualitative approach for the purpose of species authentication, evaluation of quality and ensuring the consistency and stability of herbal drugs and their related products. In the present research article TLC and HPLC Chromatogram of *Aegle marmelos* Corr is taken as standard for comparing its fingerprinting profile with three marketed formulations containing *Aegle marmelos* Corr as one ingredient. Bilva patra showed four spots at 0.89, 0.75, 0.65, and 0.52. in thin layer chromatography. Bilwadi churna also showed three spots at 0.89 ,0.80 and 0.63. Pushyanug churna showed two spots at 0.89 and 0. .Whereas Gangadhar churna showed two spots of which only one spot is matching with Bilva Patra (0.63). On the inspection of various chromatogram of Bilva Patra and its formulations in Polyherbal drugs, gives various peaks from 4.8-10.. It seems that the peaks at 4.8,5.8,and 9.2 may be beneficial for standardizing the Bilva Patra in the polyherbal formulations,but when the chromatogram of polyherbal formulations have been been recorded , it was found that the peaks obtained at 9.2 have been retarded to a negligible amount, but only the peak of 5.8 min can be taken as standard.

Keywords: *Aegle marmelos* Corr , Bilva Patra , Bilwadi churna, Pushyanug churna, Gangadhar churna

Introduction

Herbal medicines have a long therapeutic history and are still serving many of the health needs of a large population of the world. But the quality control and quality assurance still remains a challenge because of the high variability of chemical components involved. Herbal drugs, singularly and in combinations, contain a myriad of compounds in complex matrices in which no single active constituent is responsible for the overall efficacy. This creates a challenge in establishing quality control standards for raw materials and standardization of finished herbal drugs. Traditionally only a few markers of pharmacologically active constituents were employed to assess the quality and authenticity of complex herbal medicines.[1] Chromatographic fingerprint analysis of herbal drugs represents a comprehensive qualitative approach for the purpose of species authentication, evaluation of quality and ensuring the consistency and stability of herbal drugs and their related products.[2]Recent advances in the isolation, purification, and structure elucidation of naturally occurring metabolites have made it possible to establish appropriate strategies for the determination and analysis of quality and the process of standardization of Ayurvedic preparations.. The results from these sophisticated techniques provide a chemical fingerprint as to the nature of chemicals or impurities present in the plant or extract.[3]High Performance Liquid Chromatography (HPLC) is one mode of chromatography, one of the most used analytical techniques.[4] HPLC has over the past decade become the method of choice for the analysis of a wide variety of compounds.[5] Aegle marmelos Corr. (syn. *Craterva marmelos* L.) which belongs to the Rutaceae family, commonly named as Bael in Hindi found abundantly all over India. Almost every part of the plant is used in traditional medicine.**[6]** Skimmianine, Aegeline, Lupeol, Cineol, Citral, Citronella, Cuminaldehyde, Eugenol, Marmesinine are the main phytoconstituents of leaves [7]Leaves are applied to inflammation parts .Juice of fresh leaves has a laxative action and also employed in asthmatic complaints, ophthalmic and other eye affections.[8] Decoction of leaves is used as a febrifuge and expectorant. Medicated oil prepared from leaves gives relief from recurrent cold and respiratory infections.[9] Leaves are also use in Abscess, backache, abdominal disorders, vomiting, cut and wounds, dropsy, beriberi, weakness of heart, cholera, diarrhea, cardio tonic, blood sugar, injuries caused by animals, nervous disorders, hair tonic, acute bronchitis, child birth.**[10-12]** The main objective of the present study is focused on identification of *Aegle marmelos* Corr (Bilva patra) present in polyherbal formulations based on TLC and HPLC fingerprinting profiles. In the present research article TLC and HPLC Chromatogram of *Aegle marmelos* Corr (Bilva patra) is taken as standard for comparing its fingerprinting profile with three marketed formulation These formulations contain *Aegle marmelos* Corr (Bilva patra) as one ingredient. In this research work qualitative analysis was done based on the fingerprinting profile of standard plant in comparison with its formulations.

Materials and methods

Chemicals and Reagents

The entire chemicals used in the experiment were of analytical grade. All the solvents used in the experiment were procured from RFCL Pvt.LTD, New Delhi, India.

Procurement Of Crude Drug:

The crude drug i.e dried leaves of *Aegle marmelos* Corr. (Fig No.1) was purchased from the local crude drug shop, Jagram ganga sahay store in Jaipur, Rajasthan and their identity was confirmed by correlating their morphological and microscopical characters with those given in literature.[13,14,15]

Marketed Formulations:

The marketed formulations containing *Aegle marmelos* Corr. as one of the ingredient, were purchased from a registered Ayurvedic pharmacy in Jaipur ,Rajasthan. Three different formulations like Bilvadi Churna , Gangadhar Churna, and Pushyanug Churna .(Fig No.2)

Extraction:

The extraction was carried out in a Soxhlet apparatus. Shade dried leaves of *Aegle marmelos* Corr. were subjected for size reduction to coarse powder, and marketed formulations containing *Aegle marmelos* Corr. like Bilwadi churna, Gangadhar churna, and Pushyanug churna were extracted with 90% ethanol at 45° using soxhlet apparatus for 72 h. The ethanolic extracts of these churnas were filtered, concentrated and were used for TLC and HPLC analysis[16].

Instrumentation and chromatographic conditions

Thin Layer Chromatography

Slurry of silica gel G was prepared in distilled water and poured on glass to form a thin film. The prepared plates were allowed for setting (air-drying). After setting, the plates were kept in an oven at 100 to 120C (30 min) for activation [16]. The extracts of samples were applied to the activated plates (1cm above from the bottom). It was then kept in previously saturated developing chamber containing mobile phase, and allowed to run 3/4th of height of the plate.Mobile phase: n-Butanol : Acetic acid: Water (4:1:5) was taken in an development chamber.Mobile phase was previously saturated in a chamber, and ascending development technique is commonly used .TLC plates were placed in Iodine chamber for spot location.Rf Values were calculated by noting distance travelled by solute and solvent (Table no.1)

See Rf values in Table no.1

High performance liquid chromatography

1 ml of alcoholic extracts of *Aegle marmelos* Corr.and containing different formulations were taken and transferred to the 10ml clean and dried volumetric flask. Then the volume was made up to the mark with ethanol. From the above freshly prepared sample, 2ml was pipetted out and transferred to the clean and dried 25ml volumetric flask. Finally the volume was made up to the mark with ethanol.In the present research work, ethanolic extracts of *Aegle marmelos* Corr. containing marketed polyherbal formulations were runned through HPLC instrument having following chromatographic conditions:

Mobile phase : Ethanol : Water : Acetic acid

Column : Phenomenex C18 (250 x 4.6 mm, 5 µm) column

Detector : UV-Visible detector.

Wave length : 270 nm

Instrument name : Shimadzu SPD10-AT-VP

Injected volume : 20 µl sample loop.

Flow rate : 0.5ml/min.

The chromatograms obtained for the three marketed formulations are compared with that of *Aegle marmelos* Corr. (Table no.2).

Results and discussion

Thin Layer Chromatography

The ethanolic extracts of all the churnas were subjected for TLC and their Rf values are given in Table no.1. Bilva patra showed four spots at 0.89, 0.75, 0.65, and 0.52. Bilwadi churna also showed three spots at 0.89 ,0.80 and 0.63 matching with two phytoconstituents of Bilva Patra. Pushyanug churna showed two spots at 0.89 and 0.66 matching with phytoconstituents of Bilva Patra .Whereas Gangadhar churna showed two spots of which only one spot is matching with Bilva Patra (0.63).

HPLC Studies

On the inspection of various chromatogram of Bilva Patra(Fig no.3) and its formulations (Fig no.4,5,6) in Polyherbal drugs, gives various peaks from 4.8-10. Under RP HPLC conditions from the Review of literature it can be correlated that these peaks are from various esters of gallic acid and epigallic acid namely called as tannins. It seems that the peaks at 4.8,5.8,and 9.2 may be beneficial for standardizing the Bilva Patra in the polyherbal formulations,but when the chromatogram of polyherbal formulations have been been recorded , it was found that the peaks obtained at 9.2 have been retarded to a negligible amount, but only the peak of 5.8 min can be taken as standard.(Table no.2) From the chromatogram obtained from the polyherbal formulations ,it has been found that the peak at 5.8 have been obscured with the peak starts from 4.7 and ends at 7min, possibley due to the similar availability in the other plants as tannins are available in all the plants of polyherbal formulations.

Conclusion

On the observation of the results and discussions it can be concluded that the standardization of herb *Aegle marmelos* Corr. in formulations can be done on the basis of chromatographic patterns Quantitative analysis of the *Aegle marmelos* Corr. and three brands of marketed ayurvedic formulations having this plant as main compound showed varying patterns in HPLC chromatogram suggesting that there might be differences in their geographical distribution, process of manufacturing, time and method of collection and therefore a proper scientific validation (chromatographic fingerprinting, quantitation of major constituents) is needed for quality control purposes.

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Fig.1 Leaves of *Aegle marmelos* Corr.

Fig.2 .Three marketed formulations containing *Aegle marmelos* Corr.

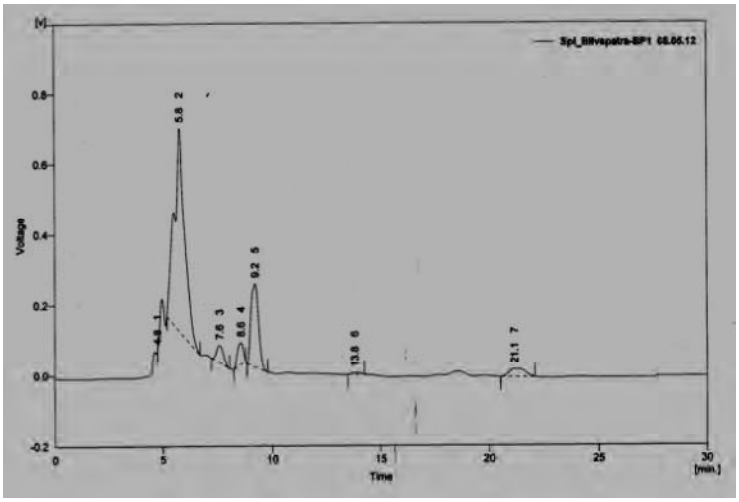

Fig no.3 : HPLC Chromatogram of Bilva patra extract.

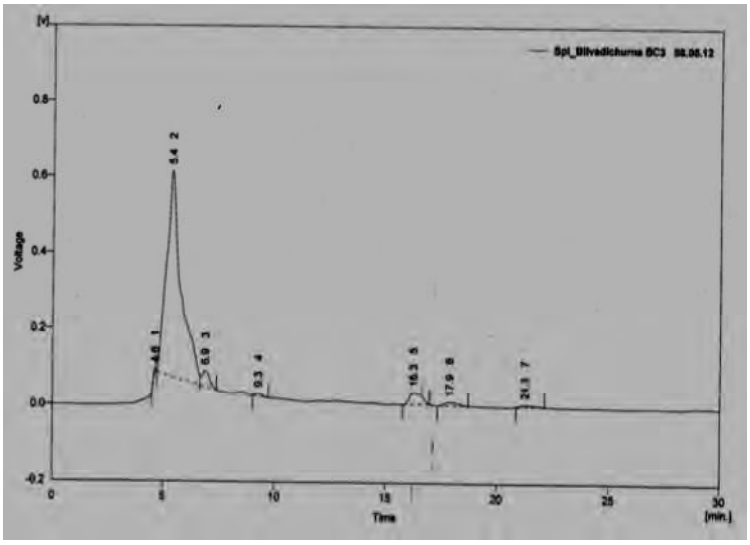

Fig no. 4: HPLC Chromatogram of Bilvadi Churna

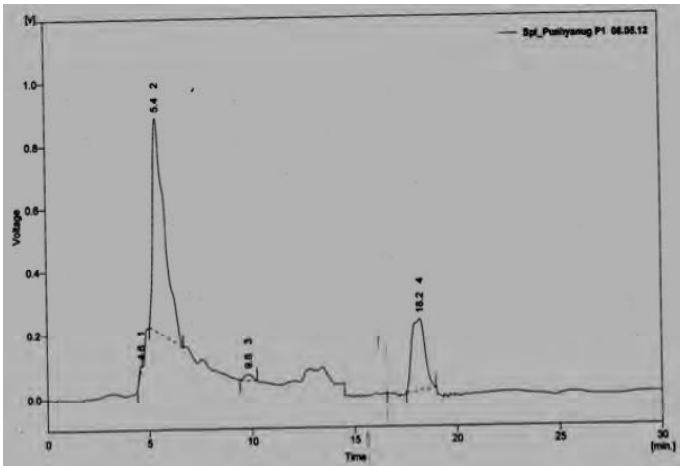

Fig no.5 : HPLC Chromatogram of Pushyanug Churna

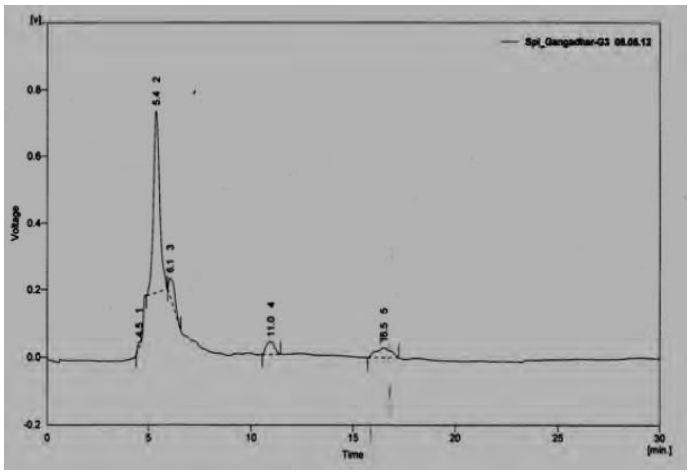

Fig no.6: HPLC Chromatogram of Gangadhar Churna.

Table No.1 : TLC of the Bilva Patra and their Formulations R_f values

S No.	Formulations		R_f Values
1	Bilva patra	Spot 1	0.89
		Spot 2	0.75
		Spot 3	0.65
		Spot 4	0.52
2	Bilwadi churna	Spot 1	0.89
		Spot 2	0.80
		Spot 3	0.63
3	Pushyanug churna	Spot 1	0.89
		Spot 2	0.66
4	Gangadhar churna	Spot 1	0.63
		Spot 2	0.40

Tabel no.2 Comparative HPLC studies of Phytoconstituents

R_T Range	**Bilva patra**		**Bilvadi churna**		**Pushyanug churna**		**Gangadhar churna**	
	R_T	**Area**	**R_T**	**Area**	**R_T**	**Area**	**R_T**	**Area**
4-5	4.758	2.236	4.643	0.44	4.627	0.40	4.543	0.90
5-6	5.800	67.97	5.390	88.97	5.425	72.30	5.363	74.77
6-7	-	-	6.903	3.52	-	-	6.057	8.41
7-8	7.602	3.75	-	-	-	-	-	-
8-9	8.577	4.01	-	-	-	-	-	-
9-10	9.230	19.91	9.270	0.59	9.918	1.29	-	-
10-11	-	-	-	-	-	-	10.970	6.49
13-14	13.800	0.47	-	-	-	-	-	-
16-17	-	-	16.255	4.35	-	-	16.517	9.44
17-18	-	-	17.872	1.35	-	-	-	-
18-19	-	-	-	-	18.232	26	-	-
21-22	21.143	3.89	21.258	0.78	-	-	-	-