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HPTLC Fingerprint Analysis of Herbs with Nutraceutical Potentials: *Amaranthus tricolor* and *Amaranthus viridis*

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Abstract
Background: *Amaranthus tricolor* and *Amaranthus viridis* have wide distribution in India as well as South Africa, also used as pseudo-cereals in Europe and America due to their nutraceutical potentials. These herbs were used in preparation of Indian traditional medicines for treatment of wide spectrum of diseases and disorders.
Objectives: To establish HPTLC Fingerprint analysis of chloroform extract of *Amaranthus tricolor* and *Amaranthus viridis* herbs.
Materials and Methods: The chloroform extracts of *A. viridis* (AVC) and *A. Tricolor* (ATC) were selected for HPTLC analysis. The best resolutions were obtained with solvent system of toluene: ethyl acetate in ratio of 7:3. CAMAG Linomat 5 applicator was used for sample application in volume of 5 and 10 micro litres for both plant extracts AVC and ATC in the form of band on precoated plates. The plates were developed in CAMAG glass Twin Trough Chamber 20x10cm in linear ascending direction. The total numbers of tracks were four. The detection of spots were carried out by using CAMAG TLC Scanner-3 with mercury remission florescence lamps at wavelength of 366 nm. The retention factor (Rf) values and finger print data were recorded by WIN CATS planer chromatography manager software.
Results: The result revealed presence of thirteen components in *A. viridis* extract (AVC) and fourteen in *A. Tricolor extract* (ATC).
Conclusion: The main finding of present study was that HPTLC finger printing of *A. viridis* and *A. Tricolor* chloroform extracts were first time established. In future these finding could be helpful for herbal drug standardization.

Keywords: *Amaranthus tricolor, Amaranthus viridis*, Fingerprinting, HPTLC

Introduction
The use of chromatographic systems allows the separation of natural compounds with various selectivities, like separation of flavonoids, phenolic acids, alkaloids, coumarins and other phytochemicals. Fingerprint methods for TLC and HPTLC are commonly used to obtain preliminary information about the chemical composition of plant extracts and for the identification of selected plant species. For many plant extracts, fingerprint profiles were obtained [1, 2].
Amaranthus tricolor (Family-Amaranthaceae) purple red colour leafy vegetable consumed as nutraceutical herb in Bihar, Jharkhand and West Bengal. It has wide distribution in India as well as South Africa also used as pseudo-cereals in Europe and America. It has been used for the treatment of piles, blood disorders, bladder distress, tooth ache, dysentery and as astringent, diuretic, haemorrhage and hepatoprotective agent [3].
Amaranthus viridis L. (Family: Amaranthaceae), a common spinach, has been used in traditional Indian medicine to reduce labour pain and as an antipyretic [4]. Amaranthus herbs like specie of viridis, commonly called Karund or jangali Chowlai or Chilaka thotakura inTelugu, is reported to have very high nutritious value. It contains vitamins (A, B_1, C, B_2), phosphorus, calcium, iron, amino acids etc. It is frequently use in soups in Nigeria (called Alayyahu), and traditionally eaten in South India as leafy vegetable as *bhaji* a famous favourite Indian dish [5].
The aim of present research is to receive HPTLC finger print profile for selected nutraceuticals herbs of Amaranthus species: *Amaranthus tricolor* and *Amaranthus viridis* chloroform plant extracts.

Materials and methods

Plant materials and collection Collection

Identification and Pre-treatment of Plant Material Fully matured herbs of *Amaranthus viridis* were collected in the month of February 2017 from naturally grown field of Allahabad district, UP and entire herb of *Amaranthus tricolor* from Jharkhand, India. These plants were properly identified by experts of Pharmacognosy in Department of Pharmaceutical Sciences, Faculty of Health Sciences, SHUATS, Allahabad. Plants genuineness were confirmed and authenticated. Both plants material were separately exposed to dry at room temperature, crushed to powder and subjected to standardizations and preliminary phytochemical investigations.

Preparation of plant extracts

Successive solvent extraction (continuous hot percolation) technique was employed by using Soxhlet apparatus, with the use of petroleum ether (65-85 °C), chloroform and ethanol in successive section. All solvents were used of laboratory grade. The Solvents were recovered by distillation. Petroleum ether soluble extractable were yellowish in colour for *A. viridis* and *A. Tricolor* respectively. Both the extracts were dark green in case of chloroform soluble extractable and dark green colour of ethanol soluble extractable, were obtained for *A. viridis* and *A. Tricolor*. The percentage yield of petroleum ether (65-85 °C), chloroform and ethanol were recorded.

HPTLC fingerprinting

Development of solvent system

In the present study chloroform extracts of *A. viridis* (AVC) and *A. Tricolor* (ATC) were selected, based on TLC fingerprinting analysis by use of various solvent systems. The chloroform extracts of both the herbs were tried through TLC in repetitive adjusted solvent proportions, in attempt to develop standard suitable solvent system that could be capable of separating the individual phyto-components of the plant samples. The best resolutions were obtained with solvent system of toluene: ethyl acetate in ratio of 7:3.

Sample application and development

The sample applications of plant extract were performed with CAMAG Linomat 5 applicator. The total numbers of tracks were four, with application volume of 5 and 10 micro litres for both plant extracts AVC and ATC. Application of samples summarize in given

Table 1: Application of samples

S.N	Application position	Application volume	Sample ID (Plant extract)	Activity
1	15.0 mm	5.0 µl	(AVC)	Yes
2	38.3 mm	10.0 µl	(AVC)	Yes
3	61.6 mm	5.0 µl	(ATC)	Yes
4	84.9 mm	10.0 µl	(ATC)	Yes

The samples were spotted with 100 µl syringe in the form of band on precoated plates. The plates were developed in CAMAG glass Twin Trough Chamber 20x10cm in linear ascending direction with solvents system of toluene: ethyl acetate in ratio of 7:3.The total number of tracks were four, of 23.3 mm apart each spot.

Detection

Developed plates were dried in hot air oven at 60°C for 5 minutes for evaporation of solvents. The detection of spots were carried out by using CAMAG TLC Scanner-3 with mercury remission florescence lamps at wavelength of 366 nm. The plates were placed inside illumination instrument or photo-documentation chamber of CAMAG Reprostar 3 and captured the images with digital camera under ultra violet light at 366 nm scanning wavelength. The retention factor (Rf) values and finger print data were recorded by WIN CATS planer chromatography manager software.

Result

The HPTLC fingerprinting of chloroform extracts of *A. viridis* and *A. Tricolor* by using solvent system of toluene: ethyl acetate in ratio of 7:3 revealed successful separation of number of phtytoconstituents corresponding to retention factors (Rf) and chromatographic peaks. The result revealed presence of thirteen (13) components in *A. viridis* extract (AVC) and fourteen (14) in *A. Tricolor extract* (ATC). The number of constituent peaks and Rf values, max %, area % for *A. viridis* chloroform extract (AVC) were summarised in table 2.

Table 2: AVC numbers of constituents (peaks) and their Rf values

Peak	Start position Rf	Start position Height	Max Rf	Max Height	Max %	End position Rf	End position Height	Area	Area %	Assigned substance
1	0.07	0.8	0.10	24.0	1.63	0.12	14.5	427.0	1.15	unknown *
2	0.12	14.6	0.14	83.2	5.66	0.19	5.0	2264.2	6.09	unknown *
3	0.19	5.0	0.22	10.7	0.73	0.23	0.2	180.6	0.49	unknown *
4	0.24	0.4	0.25	25.2	1.71	0.26	19.5	245.5	0.66	unknown *
5	0.34	10.8	0.37	28.8	1.96	0.41	19.0	1213.4	3.27	unknown *
6	0.41	19.0	0.45	65.9	4.48	0.46	58.5	1534.8	4.13	unknown *
7	0.48	63.5	0.51	106.8	7.26	0.54	41.8	3593.5	9.67	unknown *
8	0.54	41.9	0.58	168.1	11.43	0.62	40.4	4465.6	12.02	unknown *
9	0.62	42.1	0.62	54.6	3.72	0.63	35.2	406.0	1.09	unknown *
10	0.66	50.4	0.69	236.1	16.05	0.71	33.8	5203.7	14.01	unknown *
11	0.72	36.1	0.72	65.5	4.45	0.76	33.0	1573.2	4.23	unknown *
12	0.76	33.5	0.79	282.2	19.19	0.80	265.3	5294.2	14.25	unknown *
13	0.80	265.4	0.82	319.7	21.74	0.89	0.4	10748.8	28.93	unknown *

The number of constituent peaks and Rf values, max %, area % for *A. Tricolor* chloroform extract (ATC) were summarised in table 3.

Table 3: ATC numbers of constituents (peaks) and their Rf values

Peak	Start position Rf	Start Height	Max Rf	Max Height	Max %	End position Rf	End position Height	Area	Area %	Assigned substance
1	0.00	6.3	0.01	281.2	12.30	0.04	3.1	3245.9	5.35	unknown *
2	0.05	0.3	0.06	14.0	0.61	0.07	0.3	109.9	0.18	unknown *
3	0.08	0.0	0.11	55.0	2.41	0.13	13.9	872.1	1.44	unknown *
4	0.13	14.0	0.16	136.0	5.95	0.21	1.8	3137.8	5.17	unknown *
5	0.21	2.1	0.21	19.9	0.87	0.24	1.8	176.1	0.29	unknown *
6	0.26	6.2	0.29	32.0	1.40	0.31	24.3	887.0	1.46	unknown *
7	0.35	26.7	0.37	49.1	2.15	0.39	0.3	983.4	1.62	unknown *
8	0.39	0.3	0.42	44.9	1.96	0.43	43.7	741.1	1.22	unknown *
9	0.46	74.0	0.50	237.7	10.39	0.56	72.4	11443.3	18.87	unknown *
10	0.56	73.0	0.59	224.4	9.81	0.63	69.0	6673.6	11.00	unknown *
11	0.63	69.4	0.69	418.7	18.31	0.72	62.8	12149.6	20.03	unknown *
12	0.72	63.9	0.74	116.7	5.10	0.77	52.6	2705.5	4.46	unknown *
13	0.77	53.2	0.79	272.5	11.91	0.81	205.3	5432.6	8.96	unknown *
14	0.81	207.2	0.84	385.0	16.83	0.89	1.5	12097.1	19.94	unknown *

The HPTLC chromatogram of AVC shown by figure:1 and HPTLC chromatogram of ATC shown by Figure: 2.

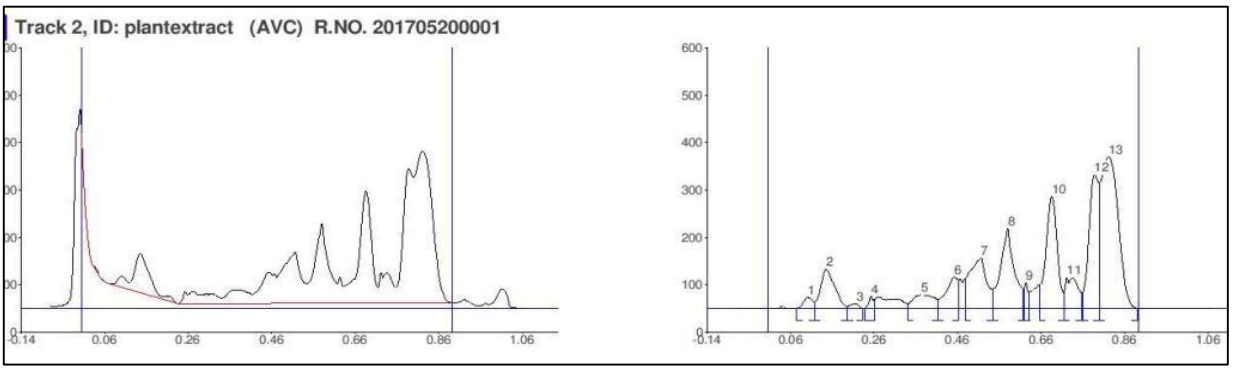

Fig 1: HPTLC chromatogram of AVC

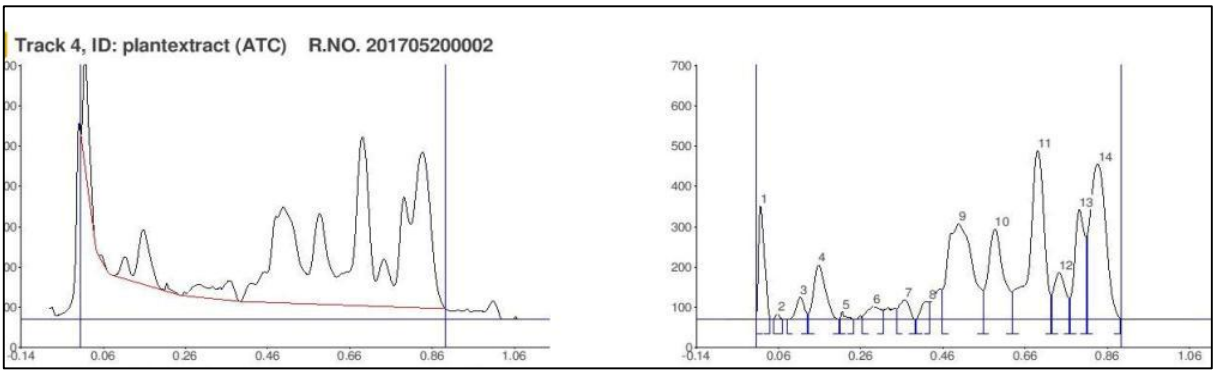

Fig 2: HPTLC chromatogram of ATC

Chromatographic profile for AVC and ATC with single and double volume of extract samples, under 366nm shown by figure: 3.

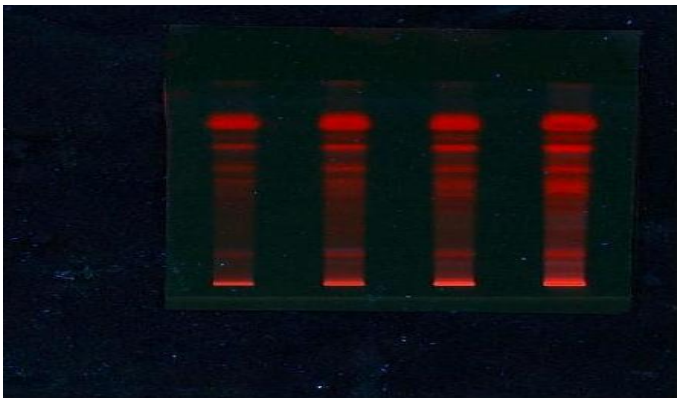

Fig 3: Chromatographic profiles of ATC and AVC under 366nm

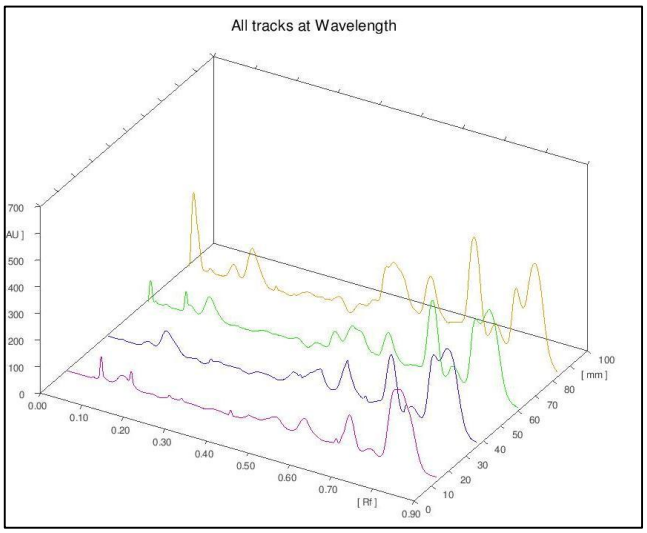

Fig 4: Three dimensional representation of all tracks at different wavelength.

Graphical representation of all the four tracks with their Rf and area at different wavelengths shown by figure: 4.

Discussion

These two selected plants *A. viridis* and *A. Tricolor*, belongs to taxa Amaranthaceae. As per the previous report on these plants, showed that all of investigated taxa have flavonoid sulphate, flavon *C* and *C-/O* glycosides and aglycons in their root and aerial parts with the exception of leaves that had not aglycons. Isorhamnetin, Kaempferol, Quercetin and Rutin were found in all of studied taxa aerial parts. All of taxa roots had kaempferol, quercetin and rutin. It is believed that plant color is directly or indirectly correlated with secondary metabolites specially flavonoids and anthocyanins and other reported compounds were squalene and polysaccraides[3]. The above selected solvent system based on reports on flavaniods supporting solvent system. So it may be strong possibility of presence of numbers of flavonoids in the selected ATC and AVC extracts. The above HPTLC finger printing profile of the selected plants showed good similarity in their components. So it could be possibility of presence of similar nature of phyto constituents in plants *A. viridis* and *A. Tricolor*.

Conclusion

The HPTLC finger printing profile of the plants *A. viridis* and *A. Tricolor* indicated that these contain appreciable number of active phytoconstituents in their chloroform extracts. These finding supports identity and purity of herbal these plants as well as helpful for detection of any adulterant plant species. The main finding of present study was that HPTLC finger printing of *A. viridis* and *A. Tricolor* chloroform extracts were first time established. In future these finding could be helpful for herbal drug standardization. *A. viridis* and *A. Tricolor* are common weeds and consumed as edible leafy vegetable, in various parts of the world as well as in India, due to its nutritive values. So these plants could be explored for cheap source of herbal medication and formulations for wide spectrum of diseases.

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