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Preliminary phytochemical investigation and TLC fingerprint profile of *Amaranthus* herbs with nutraceutical potential

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Abstract

Background: *Amaranthus viridis* and *Amaranthus tricolor* were herbs with nutraceutical potentials. These herbs were consumed as leafy vegetable in various parts of India as cheap and nutritive source of food materials among low economic society.

Objectives: Present research focused on TLC fingerprinting of selected herbs *Amaranthus viridis* and *Amaranthus tricolor*.

Materials and methods: Petroleum ether, chloroform and ethanolic extracts of, both the selected herbs were used for TLC finger printing. Standard rutin was used for detection of flavonoids. Silica gel-G used as stationary phase and combinations of solvent system were used as mobile phase, in linear ascending direction in chromatographic chamber and detection was carried out under UV chamber.

Result: The result revealed presence of appreciable number of phyto chemical compounds corresponding to RF values along with flavonoids compound rutin.

Conclusion: The result of the study suggested positive evidence about plant extracts of *A. viridis* and *A. tricolor* as nutraceutical and pharmaceuticals.

Keywords: TLC fingerprinting, nutraceuticals, flavonoids, phytochemicals, *A. tricolor*, *A. viridis*

Introduction

Dual benefit obtained from taking vegetable plants as food and overlapping medicine, always referred as nutraceuticals value, is an ancient traditional practice known for centuries [1]. *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 [2]. *Amaranthus viridis* L. (Family: Amaranthaceae), a common spinach, has been used in traditional Indian medicine to reduce labour pain and as an antipyretic. Amaranthus herbs like specie of *viridis*, commonly called Karund or jangali Chowlai or Chilaka thotakura in Telugu, is reported to have very high nutritious value. It contains vitamins (A, B₁, C, B₂), 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 [3]. This plant has been reported to have a high concentration of antioxidant components [4] and it contains quercetin, an important constituent, in large amount [5]. It also possesses antidiabetic, antihyperlipidaemic and antioxidant activities in experimentally induced diabetes [6]. Leaf and seed extracts of *Amaranthus viridis* also reported to have antioxidant and antimicrobial activities [7].

TLC Fingerprint is a vital analytical technique found very effective for separation, identification and estimation of different classes of phyto- compounds. The various phytopharmacological potentials of the *Amaranthus tricolor* reported were antioxidant, antimicrobial, Anti-nociceptive and anti-inflammatory activity, Tumor cell proliferation and cyclooxygenase enzyme inhibitory compounds, Hematological, Hypoglycemic, Hypolipidemic, Hepatoprotective activity [8].

Present research focused on TLC fingerprinting of selected herbs *Amaranthus viridis* and *Amaranthus tricolor*.

Materials and Methods

Petroleum ether, chloroform and ethanolic extracts of, both the selected herbs were used for TLC finger printing. Standard rutin was used for detection of flavonoids. Silica gel-G used as stationary phase and combinations of solvent system were used as mobile phase, in linear ascending direction in chromatographic chamber and detection was carried out under UV chamber.

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 *Amaranthus tricolor* from Jharkhand, India. These plants were identified by experts in Pharmacognosy Department of Pharmaceutical Sciences, Faculty of Pharmacy, Shalom Institute of Health and Allied Sciences, SHUATS. Plants genuineness were confirmed and authenticated. Both plants material were separately exposed to dry at room temperature, crushed to pieces with bare hands and their weight recorded.

Preparation of Plant Extracts

Successive solvent extraction (continuous hot percolation) technique was employed using Soxhlet apparatus and cold maceration for aqueous extract as per WHO guidelines. Solvents and all chemicals used were analytical grade.

Petroleum ether soluble extractives

The dried coarsely sized herbs of *A. viridis* and *A. tricolor* each weighed approximately 400g were separately extracted with Petroleum ether (65-85 °C) until maximum extracts were obtained. The Solvent was recovered by distillation. The greenish and yellowish extracts obtained respectively for the *A. viridis* and *A. tricolor* slightly reflects the colour of their marc. The percentage yields of the extracts were noted and extracts were finally stored for subsequent analytical use.

Chloroform soluble extractives

The respective marc left after petroleum ether extraction were separately dried and then subjected for extraction with chloroform (54-58 °C). After the extraction was finished, the chloroform solvent was recovered by distillation. Both the extracts were dark green in colour and stored for future use. Percentage yield were recorded.

Ethanol soluble extractives

Similarly, the dried left behind marc of chloroform extraction were further extracted with ethanol 95% v/v (75-78 °C). After completion, the solvent was removed by distillation. Pale yellowish green extracts were obtained for both *A. viridis* and *A. tricolor* and their percentage yield recorded.

Water soluble extractives

Similarly, the dried left behind marc of ethanol extraction were further extracted with chloroform water by cold maceration and extractive value calculated.

Preliminary Phytochemical Screening

The phytochemical investigation of the crude petroleum ether, chloroform, ethanol and aqueous extracts of *A. viridis* and *A. tricolor* were performed by standard methods. The detection of glycosides, alkaloids, phenolic compounds and tannins, saponins, flavonoids, resins, proteins and amino acids, fixed oil and fats, carbohydrates, sterols, were performed as per standard methods [9].

Thin Layer Chromatography (TLC) Fingerprint Studies

The principle of separation in TLC is basically adsorption, particularly when Silica gel G is used as stationary phase, and partly with partition. Mobile phase solvent typically flows through the stationary phase because of capillary action. The components move and get separated according to their affinities towards the adsorbent. The component having more or less affinity towards the stationary phase travels slower or faster respectively. TLC constantly proves useful in studies, and assessing quality and purity, of herbal medicines, generally due to its simplicity, cost effectiveness, and speed. TLC studies carried out for different extracts with different solvent systems of different polarity indices in different ratios. This attempt helps to precisely acknowledge the solvent system capable of showing best resolution in separating the various components of the sample plant under investigation. The adsorbent used was silica gel G.

Preparation of Slurry and Plates

About 150g of Silica gel G was weighed and mixed well with about 250ml distilled water to form slurry. The slurry was poured and spread in a thin uniform layer on to the already washed dried TLC plates. The plates were allowed to air dry for approximately 20min after which they were subjected for activation in hot air oven at 100-105 °C for 1hr. The prepared plates were properly stored for future use. Prior to use, they were shallowly reactivated in same hot air oven at 100 °C for 10min.

Sample preparation

Small quantities of the sample extracts of *Amaranthus viridis* and *Amaranthus tricolor* were taken and dissolved in their respective solvents. The selected plant extracts were pet ether, chloroform and ethanol.

Preparation of standard pure Rutin

10mg of standard pure rutin was dissolved in 1ml methanol. This was used for spotting on the already prepared activated TLC plates.

Comparative TLC Study

To justify presence of a specific flavonoid compound in the sample herbs, rutin was selected for the comparative study. The selection of marker compound rutin based on previous literature available on same plant species.

The above prepared sample extracts solutions were applied on the prepared TLC plates using capillary tubes and immersed in presaturated TLC development chamber containing suitable solvent system as mobile phase, covered and allowed to develop. The developed TLC plates were air dried and observed under UV chamber. They were later sprayed with spraying reagent and were placed in hot air oven for 1min for the development of colour in separated bands. The Rf values were calculated for each different spot observed.

$$R_f \text{ value} = \frac{\text{Distance travelled by the solute front}}{\text{Distance travelled by the solvent front}}$$

Solvent Systems

A number of combinations of solvents in different proportions were tried in attempt to develop standard solvent system capable of separating the components best in perfect resolution.

Results and Discussion

Both the herbs were subjected to successive solvent extraction with Pet ether, Chloroform and ethanol and their extractive

value were calculated as per standard methods of WHO. Results were represented in table 1.

Table 1: Percentage yield from different solvent extracts of the sample herbs

Solvent	<i>Amaranthus viridis</i>			<i>Amaranthus tricolor</i>		
	Extract colour	Yield (gm)	% Yield	Extract colour	Yield (gm)	% Yield
Pet ether	Greenish	0.57	0.57%	Yellowish	1.04	0.26%
Chloroform	Dark green	1.30	0.33%	Dark green	1.35	0.34%
Ethanol	Yellowish green	3.68	0.92%	Yellowish green	3.56	0.89%

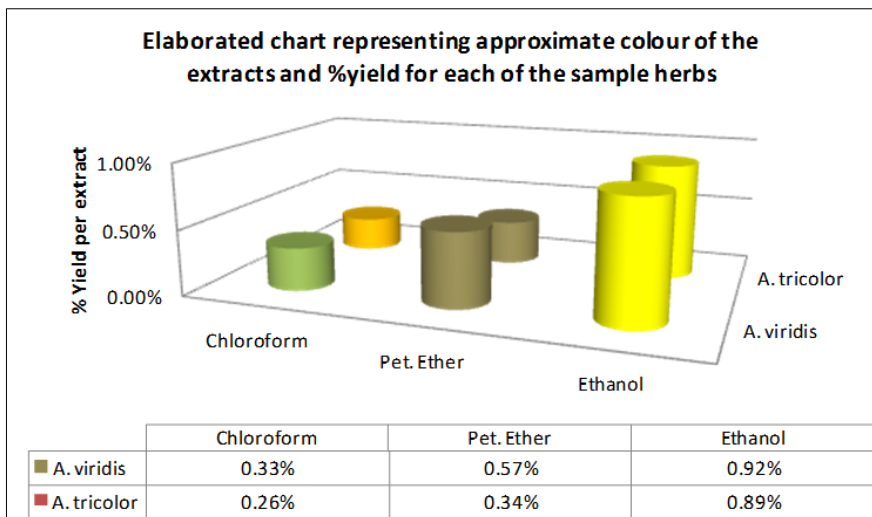


Fig 1: graphical representation of % yield of extracts

The preliminary phytochemical screenings were performed for detection of a particular class of substances using the

selected solvent extracts. The results for phytochemical screening were represented as table 2.

Table 2: Preliminary phytochemical screenings of *A. viridis* and *A. Tricolor* extract.

SN	Group test of phytoconstituents	Pet. Ether		Chloroform		Ethanol	
		A.V	A.T	A.V	A.T	A.V	A.T
1.	Glycosides	++	++	++	++	++	++
2.	Alkaloids	+++	++	+++	++	+++	-
3.	Phenolic comp. & Tannins	-	-	++	++	+++	+++
4.	Flavonoids	+++	+++	+++	+++	+++	+++
5.	Saponins	+	+	+	+	+	+
6.	Resins	++	-	++	++	++	++
7.	Fixed oil & Fat	-	-	-	-	-	-
8.	Phytosterols	++	++	++	++	++	++

(+++)+Strongly positive, (++) Moderately positive, (+) very less positive, (-) Negative

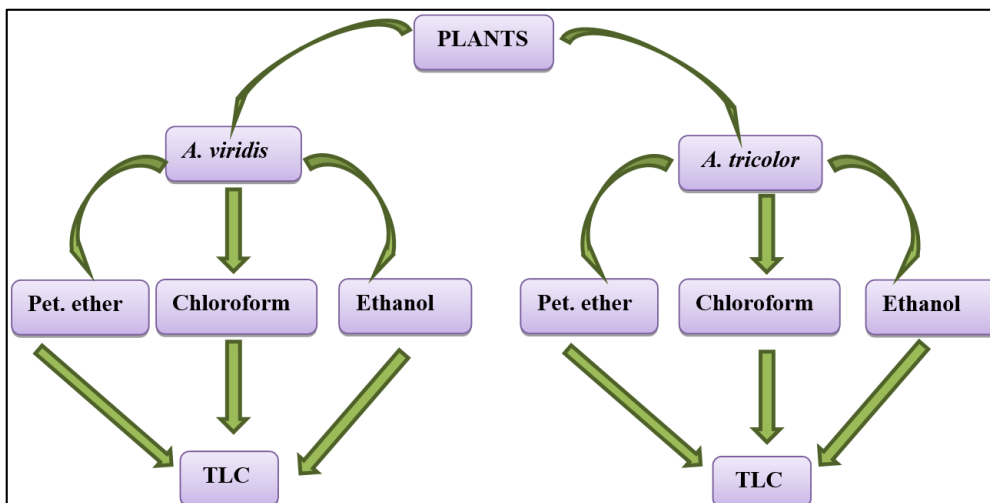


Fig 2: A flow chart briefing successive steps in the procession of the plants for TLC study

As presented above, this research study employed three different solvents Pet. ether, chloroform and ethanol, utilised to make six different extracts of the sample herbs (*A. viridis* and *A. tricolor*). All the six extracts were subjected for TLC profiling study.

Development of Suitable Solvent System

Various solvent systems in repetitive adjusted solvent proportions were tried in attempt to develop standard suitable solvent system, capable of separating the individual components of the sample herbs, in to differentiable visible spots of good resolution.

At first, solvent system ethyl acetate: butanol: acetic acid: water (80:10:5:5) was tried for all the six sample extracts (pet ether, chloroform and ethanol extracts of *A. viridis* and *A. tricolor*), but none of the extracts showed movement nor separation of spots. Solvent system, toluene: ethyl acetate

(7:3) was then tried, and separation of appreciable number of individually separated phytoconstituents in different Rf values were observed for only four (pet ether and chloroform extracts of the sample herbs) out of the six extracts. This led to further trial for only the ethanol extract of the sample herbs. Solvent system methanol: chloroform (50:50) was tried for the two ethanol extracts of the herbs, and this showed movement with no resolution (perfect individual separation of spots). Hence, the proportion of the solvents in the system was adjusted to methanol: chloroform (7:3) and few drops of formic acid (to aid good clear resolution), but finally still no appreciable sound result was obtained. Consequently, this research study was closely and only maintained for pet ether and chloroform extracts of *A. viridis* and *A. tricolor* (four extracts total) for which perfect solvent system was developed, thereby ignoring the ethanol extracts of the sample herbs.

Table 3: Optimisation of solvent system for TLC

Extracts Solvent System	Pet ether		Chloroform		Ethanol	
	<i>A. v</i>	<i>A. t</i>	<i>A. v</i>	<i>A. t</i>	<i>A. v</i>	<i>A. t</i>
I	Unsatisfactory	Unsatisfactory	Unsatisfactory	Unsatisfactory	Unsatisfactory	Unsatisfactory
II	Perfect	Perfect	Perfect	Perfect	Very Poor	Very Poor
III	—	—	—	—	Poor	Poor
IV	—	—	—	—	Imperfect	Imperfect

(I) = ethyl acetate: butanol: acetic acid: water (80:10:5:5) (II) = toluene: ethyl acetate (7:3) (III) = methanol: chloroform (50:50) (IV) = methanol: chloroform (7:3) + formic acid drops

A good TLC result based on number of spots separated and the intensity of the resolution. The result of this study depicted in the table below shows the number, colour

intensity, Rf. value and diameter of each of the spots separated in the selected solvent extracts over TLC plates.

Table 4: Respective Rf values, diameter and colour for each of the spots in the TLC studies of the herbs.

Extracts Sample herb	Pet ether		Chloroform	
	<i>A. v</i>	<i>A. t</i>	<i>A. v</i>	<i>A. t</i>
No of spots	4	3	7	7
Rf values	0.98, 0.89, 0.67, 0.19	0.89, 0.77, 0.43	0.92, 0.84, 0.68, 0.50, 0.28, 0.18, 0.12	0.96, 0.85, 0.66, 0.52, 0.30, 0.22, 0.12
Diameter of spot (mm)	7.00, 13.00, 9.00, 7.00	3.50, 4.00, 3.00	6.00, 15.00, 10.00, 8.00, 6.00, 3.50, 3.50	6.00, 15.00, 10.50, 8.50, 5.50, 3.00, 3.50
Colour of respective spot	Red, Red, Red, Pink	Red, Red, Pink	Red, Red, Red, Pink, Blue, Pink, Blue	Pink, Red, Red, Pink, Blue, Pink, Blue

The above tabulated values were explained by graphical representation.

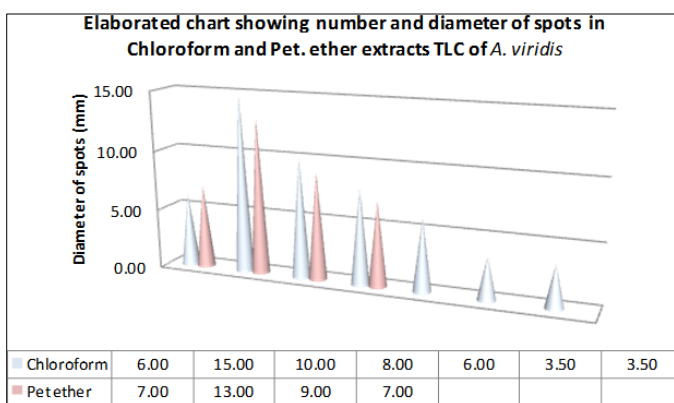


Fig 3: The number and diameter of spots in Chloroform and Pet. ether extracts TLC of *A. viridis*

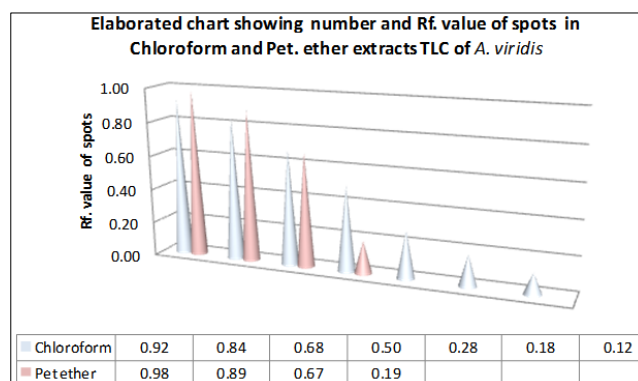


Fig 4: The number and Rf. value of spots in Chloroform and Pet. ether extracts TLC of *A. viridis*

Note that each of the peaks above represents a spot and both charts are comparable in the sense that the first peak in each chart is same spot represented and that applied to the rest of the peaks as well.

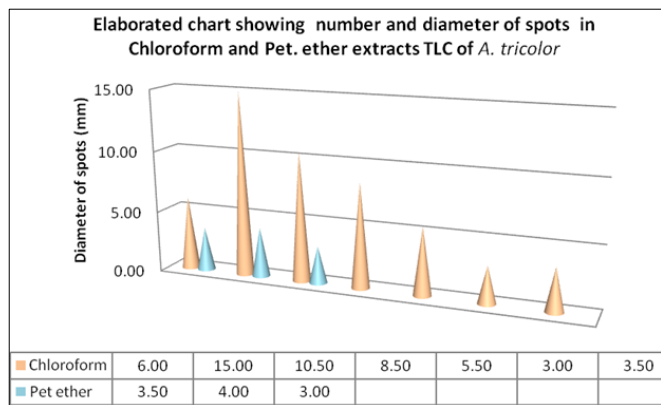


Fig 5: The number and diameter of spots in Chloroform and Pet. ether extracts TLC of *A. tricolor*

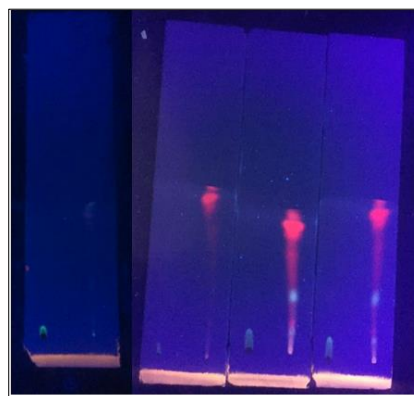


Fig 7: TLC Result pictures snapped using camera (16:9 resolution of 1334x750), showing separation of spots from sample herbs extracts with corresponding Rutin sample.

From right to left; *A.v* chloroform, *A.t* chloroform, *A. v* pet ether and *A. t* pet ether with corresponding pure Rutin sample spot ~3cm away in each.

As shown previously, the diameter of the separated spots represent relative abundance or amount of such phytochemical expressed by the spot in that sample herb. Exact resemblance of sample herbs spots colour with that of Rutin sample as well as their similar Rf values, justified existence of Rutin in experimental herbs.

Conclusion

The result of present research study clearly revealed presence of various phytochemicals of therapeutic importance in the sample herbs of *A. viridis* and *A. tricolor* such as Alkaloids, Glycosides, Flavonoids plus those of food importance such as carbohydrates, vitamins and proteins, collectively justified nutraceutical potential of the herbs. In the literature review survey performed prior to this research study, evidences proved ancient utilisation of Amaranthus herbs as traditional medicine for curing various ailments including diabetes, fever, ulcer, asthma, venerable diseases and source of palatable nutritive food as well.

The TLC fingerprint profiling study of present research, justified presence of abundant flavonoid compound, among which presence of Rutin, in Amaranthus species of *viridis* and *tricolor* both. It also showed existence of a number of other separable phytochemicals as well. Chloroform extract of studied herbs remained the best extract with appreciable result compared to other solvents extract due to outstanding positive chemical tests and perfect TLC spots resolution.

Future Prospects

In future studies, a more closely focused attention can be given to isolation, purification, qualitative and quantitative analytical estimation of each of the assumed phytochemicals of the sample herbs so as to have a novel broad spectrum of activity, a more effective, safer and much targeted utilisation of the herbs, this will help upgrade the herbs level of standard in local society and increase their local production capacity which may bring about their sound valuable status in the global market.

Furthermore, the study can help to develop a combined herbal formulation with these plant extracts for synergistic potency and also a stepping stone to therapeutic lead compounds determination can be established and rational utilization of the herbs can therefore be optimal.

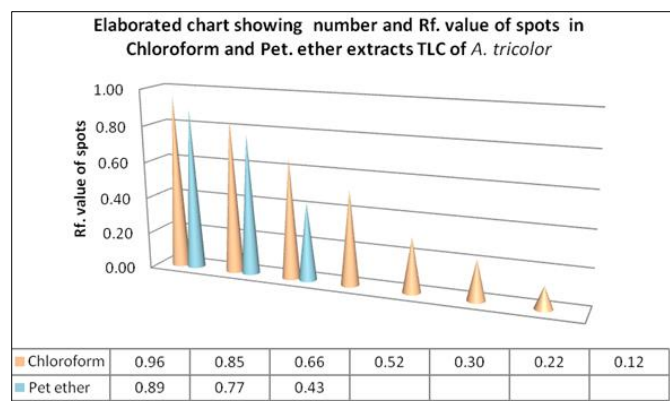


Fig 6: The number and Rf. value of spots in Chloroform and Pet. ether extracts TLC of *A. tricolor*

Rutin and Sample reading

The phytochemical investigation of the sample herbs under study revealed presence of abundant amount of phenolic compounds, tannins and flavonoids, hence this research study was extended to more specificity domain in order to precisely detect existence of Rutin (flavonoid compound) in sample herbs.

The pure Rutin sample applied on the TLC plates, which was separated at a distance of 3cm parallel from the site where the sample extract spot was placed in each of the plates for each of the extracts, was observed moving and travelled short distance along the adsorbent phase, and received single spot.

From the other side, in each TLC plate, the respective sample extracts applied travelled higher distance as much as the mobile solvent did, and numbers of individual distinguishable components got separated with different Rf values resembling exactly that obtained in perfect solvent system development trials.

Out of the separated components, spots corresponding to the pure Rutin sample spot both in physical visual colour and Rf value were observed in two out of the four extracts TLC plates, chloroform extracts of *A. viridis* and *A. tricolor*. While separation of spots were there in pet ether extract of the herbs but none closely corresponded to the pure Rutin sample spot.

Table 5: Spots Rf value of chloroform extracts of *A. viridis* and *A. tricolor* with corresponding spot Rf value of pure Rutin sample on the respective TLC plates, each have same blue colour

Chloroform extract	<i>A. viridis</i>	<i>A. tricolor</i>
Sample Rf value	0.12	0.12
Corresponding Rutin Rf value	0.11	0.12

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