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Research Article

QUANTITATIVE PHYTOCHEMICAL ANALYSIS AND HPTLC FINGERPRINTING OF *AMARANTHUS BLITUM* L. LEAVES FROM SOUTH GUJARAT

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ABSTRACT

The present investigation was aimed to carry out for the quantification of phytochemicals and HPTLC fingerprinting profile of methanolic extract of Amaranthus blitum L. leaves. The pharmacologically important phytochemicals such as alkaloids, flavonoids, saponins, terpenoids, lipid, crude fibre, chlorophyll and carotenoids were determined as per the standard procedures. The leaves were rich in flavonoids followed by saponins and alkaloids. The TLC of flavonoids also showed more number of compounds. The methanolic extract, upon HPTLC study revealed 15 numbers of different constituents with R_f values ranging from 0.08 to 0.81 in fingerprinting profile. The results of this study will aid to identity and check quality for authenticity of Amaranthus blitum L. leaves.

KEYWORDS: *Amaranthus blitum, Phytochemical, TLC, HPTLC, Methanolic extract.*

INTRODUCTION

The phytochemical word is derived from the Greek word "Phyto" means Plant; which literally shows that chemicals derived from plant. The phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attacks as well as they provide health benefits to humankind in form of medicinal ingredients and nutrients. They are not the essential nutrients and are not required by the human body for sustaining life, but have important properties to prevent or to cure some common diseases [1, 2]. They are having biological properties like antioxidant activity, antimicrobial activity, immunomoduation potential, anticancer, hormone metabolism regulation etc [3].

High performance thin layer chromatography is a form of semiautomatic instrumental TLC that provides better and advanced separation efficiency using optimized coating material, novel procedures for mobile-phase feeding, layer conditioning, and improved sample application. HPTLC generates a chromatographic fingerprint in the form of a unique sequence of peaks corresponding to the analyzed sample [4, 5].

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Amaranthus blitum L. is a green leafy vegetable belonging to Amaranthaceae family. The plant is occurred in rainy season and also in farms with other crops. It is also known as "Ukedi bhaji" and "Adbau Tandaljo" [6]. The leaves are claimed to be used in liver and kidney disorders, fever, hemorrhage, anemia. It has laxative property and is also used to enlarge spleen in hepatic disorder [7, 8]. The present study was aimed to determine the quantity of phytochemicals alkaloids, saponins, flavonoids,terpenoids,lipid, crude fibre, chlorophyll and carotenoids of *Amaranthus blitum* L. leaves. The methanolic extract was subjected to HPTLC fingerprinting study.

MATERIALS AND METHODS

Collection and authentication of plant:

Fresh leaves of the selected plants of *Amaranthus blitum* L. were collected from surrounding area of Valod, Tapi District, Gujarat, India. The plant was authenticated by NISCAIR, New Delhi with the reference no. NISCAIR/RHMD/Consult/ 2015/2872/65.The leaves were carefully washed with water to remove adherent dust, dried in shade, coarsely powdered and stored in air tight containers.

Extraction:

The powdered drug of *Amaranthus blitum* L. leaf was extracted with methanol in soxhlet apparatus. The powdered drug was weighed around 150 gm and packed in thimble of the assembly and extracted with methanol in controlled temperature. The extract was concentrated by distillation and dried on water bath.

Quantitative Phytochemical analysis:

Determination of alkaloid:

5 gm of dried leaf powder was accurately weighed into a 250 l beaker; 200 ml of 10% acetic acid in ethanol was added,

covered and allowed to stand for 4 hours. Then it was filtered and the extract was concentrated on water bath until the volume reached to one quarter of its original volume. Concentrated ammonium hydroxide was added drop by drop to the concentrated extract until the precipitation was complete. The entire solution was allowed to settle down and then washed with dilute ammonium hydroxide and filtered. The residue obtained was alkaloid, which was dried and weighed [9].

Determination of saponins:

The powdered leaf drug was weighed 20 gm and placed in a conical flask, 20% aqueous ethanol was added. The resulting suspension was heated over water bath for 4 hours with continuous stirring at 55ºC. Then filtration was done and the residue was re-extracted with fresh 200 ml 20% aqueous ethanol. The extracts were combined and reduced to 40 ml on water bath at 90ºC. The concentrated extract was cooled and transferred to 250 ml separating funnel, 20 ml diethyl ether was added and shaken vigorously. The separating funnel was allowed to settle down. Two layers were separated, aqueous and ether. The aqueous layer was collected and ether layer was discarded. This purification process was repeated three times with 20 ml diethyl ether each cycle. To the combined aqueous extract, 60 ml of n-butanol was added. The n-butanol extracts were washed twice with 10 ml 5% aqueous sodium chloride. The rest of the solution was heated on water bath. After evaporation to dryness, the samples were dried in oven up to a constant weight and the saponin content was calculated as percentage [10].

Determination of flavonoid content:

For the determination of flavonoid content, accurately weighed 10 gm of dried powdered leaf drug was extracted with 100 ml of 80% aqueous methanol at room temperature. Then the whole solution was filtered through whatman filter no.41. The extraction was repeated several times. The combined filtrates were then evaporated to complete dryness over a watrebath, cooled in desiccator and weighed up to a constant weight. The flavonoid content was calculated [11].

Determination of terpenoid content:

Accurately weighed 10 gm of powdered leaf drug was taken in a conical flask and soaked in ethyl alcohol for 24 hours. The next day it was filtered through whatman filter paper no. 41. The filtrate was then extracted with petroleum ether. The petroleum ether extract was evaporated to complete dryness and weighed. The weight of dried petroleum ether extract was taken as a measure of total terpenoid content [12].

Determination of fat or lipid content:

Two gram of dried drug powder was taken in the glass thimble kept in a flask of soxhlet extractor. The drug was extracted with petroleum ether (60-80º C) for 6- 8 hours .The extract was transferred in a pre weighed evaporating dish , evaporated to dryness and the weight of crude fat extracted was taken. The percent crude fat was calculated [13].

Determination of crude fibre content:

Crude fiber was estimated by acid-base digestion with 1.25% H_2SO_4 (w/v) and 1.25% NaOH (w/v) solutions. The residue after crude lipid extraction was weighed accurately 1 gm and placed into a beaker and 200 ml of boiling 1.25% H_2SO_4 added. The content was boiled for 30 minutes, cooled, filtered through a filter paper and residue washed with three 50 ml portions of boiling water to neutralize the solution. The drained residue was returned to the original beaker and 200 ml of boiling 1.25% NaOH added. The content was boiled for 30 minutes, filtered, washed as above, residue drained and washed with hot distilled water for neutralization. The filter paper containing the residue was dried in an oven at 130°C to constant weight and cooled in a desiccator. The residue was scrapped into pre-weighed porcelain crucible, weighed, ignited at 550-600°C in muffle furnace for 2-3 hours, cooled in a desiccator and reweighed to a constant weight. Crude fiber content was calculated and expressed as percentage loss in weigh on ignition [14].

Determination of total chlorophyll and carotenoid:

Accurately weighed 1 gm of plant leaf material was ground with 10 ml 80% acetone in mortar pestle. The mixture was kept for overnight incubation in dark. Next day it was filtered through whatman filter paper no.1 .The marc was washed out 2-3 times with 5 ml 80% acetone each time. The final volume of the filtrate was made up to 25 ml with 80% acetone. The absorbance was read at 480, 510, 663 and 645 nm. Chlorophyll *a*, Chlorophyll *b,* total chlorophyll and carotenoids were calculated using the following equations [15, 16].

$$\text{mg Chlorophyll } a \text{ / g of fresh tissue} = \frac{12.7\,(A_{663}) - 2.69\,(A_{645}) \times V}{1000} \times W$$

$$\text{mg Chlorophyll } b \text{/ g of fresh tissue} = \frac{22.9\,(A_{645}) - 4.68\,(A_{663}) \times V}{1000} \times W$$

$$\text{mg Total Chlorophyll /g of fresh tissue} = \frac{20.2\,(A_{645}) + 8.02\,(A_{663}) \times V}{1000} \times W$$

$$\text{mg Carotenoid / g of fresh tissue} = \frac{7.6\,(A_{480}) - 1.49\,(A_{510}) \times V}{1000} \times W$$

Where,

A= absorbance at specific wavelengths; V= final volume of chlorophyll content (ml); W= fresh weight of tissue extracted (g)

TLC of extracted phytochemicals:

The extracted phytochemicals; alkaloids, saponins, flavonoids and terpenoids were subjected to thin layer chromatographic studies using different solvent systems. The optimum solvent system showing maximum spots was reported after derivatization with iodine vapours. The R_f values were calculated for each spot [17, 18].

HPTLC fingerprinting profile:

HPTLC fingerprinting profile was carried out as per the method of Wagner [17] and Harborne [18] et.al.

Sample preparation:

The methanolic extract was accurately weighed 10 mg into a 10 ml volumetric flask, dissolved in methanol to make volume up to 10 ml, filtered through whatman filter paper No.42 and the filtrate was used for analysis.

Developing Solvent System:

A number of solvent systems were tried for methanolic extract and the satisfactory resolution was obtained in the solvent Hexane: Ethyl acetate (8:2).

Sample Application:

2 µl ,4 µl and 8 µl of sample solution was loaded as 6.00 mm band length on Precoated 10x10cm size plate of silica gel G_{60} F254 with layer thickness 0.2mm using a Hamilton syringe with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

Development of Chromatogram:

After the sample application, the chromatogram was developed in Twin trough glass chamber 20x 10 cm saturated with solvent Hexane: ethyl acetate (8:2) for 15 minutes.

Detection of spot:

The air dried plates were kept in a photo documentation chamber and captured the images in UV 366 nm and UV 254 nm. The chromatogram was scanned by the densitometer at 400 nm.The peak number with its height, area and R_f values of fingerprint data were recorded by WIN CATS (1.4.6 version) software.

RESULTS AND DISCUSSION

In the present study, the quantification of phytochemicals alkaloids, saponins, flavonoids, terpenoids, lipid, crude fiber, chlorophyll and carotenoids of *Amaranthus blitum* L. leaves were carried out based on standard procedures and the results are tabulated in Table 1. The alkaloids were found 7.00 ±0.577 % in leaves. The alkaloids are one of the medicinally important structurally diverse phytoconstituent having wide range of activities like antihyperyensive, antimalarial, antiarrythmatic, anticancer, stimulant and analgesic [19, 20]. Saponins are group of plant compounds that are available in forms of steroid alkaloids, glycosides of triterpenoids or steroids with hypocholesterolaemic, immunostimulant, hypoglycemic effect and anticarcinogenic properties [21]. Saponin content was found 9.67±0.881 % in leaves. Flavonoids are polyphenolic compounds that contain a C15 skeleton and possessing many pharmacological actions like antioxidant, antimicrobial, anti ageing, anti-inflammatory, anticancer and in prevention of osteoporosis [22-24]. The higher amount of flavonoid content which was found 20.00±0.577 % shows promising health benefits of the *Amaranthus blitum* L. leaves. The terpenoid content was 1.67±0.333 %. The terpenoids are group of natural products made up of isoprene units show significant pharmacological activities such as anti-viral, anti-bacterial, anti-malarial, anti-inflammatory, inhibition of cholesterol synthesis and anti-cancer activities [25]. Lipids are very good sources of energy and enhance in transport of fat soluble vitamins, insulates and protects internal tissues and contributes to important cell processes [26]. The fiber plays role in lipid metabolism and obesity control. They are helpful in diseased conditions like constipation, colon cancer and diabetes [27]. The lipid and crude fibre contents were determined in leaves were 1.96±0.008 % and 0.36±0.003% respectively. Table 2 shows the total chlorophyll content and carotenoid found in leaves were 0.502±0.014 mg/g 0.126 ±0.002 mg/g respectively. Chlorophylls are useful as photodynamic agent in tumour or cancer therapy [28]. Carotenoids act as an antioxidant and source of vitamin A activity. [29] The results of TLC in Table 3 for flavonoids showed the presence of 7 compounds having R_f values 0.06, 0.12, 0.2, 0.3, 0.36, 0.5, 0.98 in Chloroform: Methanol (9:1) solvent system revealing wide range of flavonoids in the drug. Terpenoids showed 6 compounds with R_f values 0.07, 0.1, 0.14, 0.21, 0.3, 0.36 in Toluene: Ethyl acetate (9.3:0.7) solvent system followed by 6 alkaloidal compounds in Toluene: Ethyl acetate: Diethylamine (7:2:1) having R_f values 0.34, 0.56, 0.74, 0.81, 0.89. The TLC for saponin showed a single compound with R_f value 0.93 in Chloroform: GAA: Methanol: Water (6:2:1:1) solvent system.

Table No. 1: Quantitative phytochemical analysis of *Amaranthus blitum* L. leaves

Sr. No.	Name of Phytochemical	Yield * (%)
1	Alkaloids	7.00 ±0.577
2	Saponins	9.67±0.881
3	Flavonoids	20.00±0.577
4	Terpenoids	1.67±0.333
5	Lipid	1.96±0.008
6	Crude Fibre	0.36±0.003

Values are means of three independent analyses ± SEM

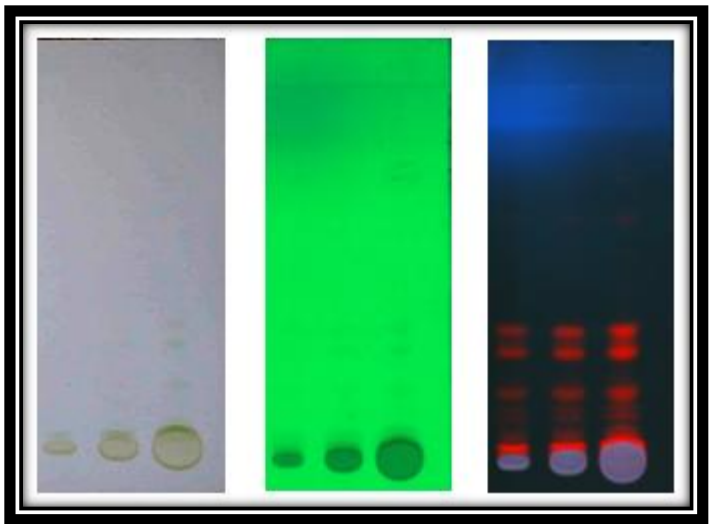
Table No. 2: Quantitative analysis of chlorophylls and carotenoid of *Amaranthus blitum* L. leaves

Sr. No.	Phytochemical	Yield* mg/g
1	Chlorophyll *a*	0.308 ± 0.002
2	Chlorophyll *b*	0.195 ± 0.013
3	Total chlorophyll	0.502 ± 0.014
4	Carotenoids	0.126 ± 0.002

Values are means of three independent analyses ± SEM

Table No. 3: TLC study of phytochemicals from *Amaranthus blitum* L. leaves

Sr. No.	Name of Phytoconstituent	Solvent system	Ratio	R_f values
1	**Alkaloids**	Toluene: Ethyl acetate: Diethylamine	7:2:1	0.34, 0.56, 0.74, 0.81, 0.89
2	**Saponins**	Chloroform: Glacial acetic acid: Methanol: Water	6:2:1:1	0.93
3	**Flavonoids**	Chloroform: Methanol	9:1	0.06, 0.12, 0.2, 0.3, 0.36, 0.5, 0.98
4	**Terpenoids**	Toluene: Ethyl acetate	9.3:0.7	0.07, 0.1,0.14, 0.21, 0.3, 0.36



Visible light UV 254 nm UV 366 nm

Fig. 1: HPTLC fingerprinting profile of Methanolic extract of *Amaranthus blitum* L. leaves

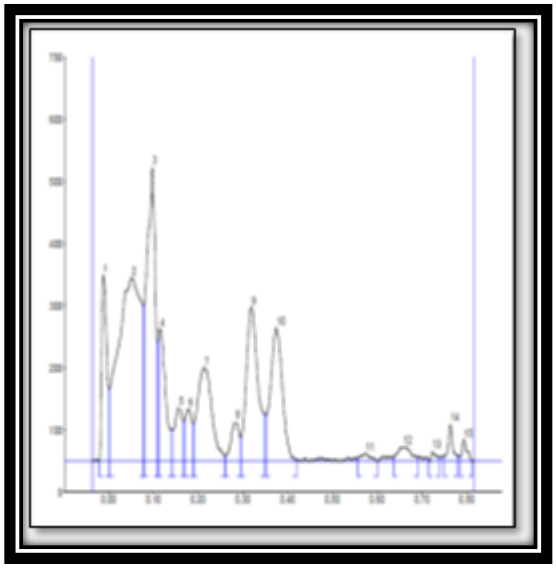


Fig. 2: HPTLC densitogram of Methanolic extract of *Amaranthus blitum* L. leaves

Table No. 4: HPTLC finger profile and Rf Values of methanolic extract of *Amaranthus blitum* L. leaves

Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	-0.02	0.7	-0.01	300.6	13.18	0.00	111.6	3313.3	6.32
2	0.00	118.1	0.05	296.3	12.99	0.08	249.8	14643.1	27.93
3	0.08	252.5	0.10	473.4	20.75	0.11	192.6	8766.8	16.72
4	0.11	197.6	0.12	213.5	9.36	0.14	50.2	3333.6	6.36
5	0.14	0.14	0.16	86.3	3.78	0.17	64.5	1538.5	2.93
6	0.17	65.2	0.18	85.1	3.73	0.19	60.9	1263.1	2.41

7	0.19	61.0	0.21	150.6	6.60	0.26	8.7	4672.3	8.91
8	0.26	9.1	0.28	64.1	2.81	0.30	37.9	1142.5	2.18
9	0.30	39.0	0.32	248.4	10.89	0.35	77.0	6219.1	11.86
10	0.35	74.5	0.38	214.7	9.41	0.42	2.2	5464.1	10.42
11	0.56	5.5	0.57	12.8	0.56	0.60	0.1	258.6	0.49
12	0.64	7.8	0.66	23.9	1.05	0.69	6.6	676.9	1.29
13	0.72	2.6	0.72	16.8	0.73	0.74	6.1	172.2	0.33
14	0.75	7.7	0.76	59.9	2.63	0.78	9.1	601.5	1.15
15	0.79	7.5	0.80	34.5	1.51	0.81	0.7	362.1	0.69

The HPTLC photodocumentation profile of methanolic extract of sample volume 8 µl of *Amaranthus blitum* L. leaves under Day light, UV 254 nm and UV 366 nm are shown in Figure 1. The HPTLC densitogram (Figure 2) and HPTLC fingerprinting profile and R_f values (Table 3) of Methanolic extract of *Amaranthus blitum* L. leaves scanned at 400 nm revealedthe presence of 15 different type of constituents in the extract. The R_f values ranged from 0.08 to 0.81. The highest concentrations out of the 15 compounds were found to be 27.93% and 16.72 % with corresponding R_f values 0.08 and 0.11 respectively.

CONCLUSION

The results of this study revealed the presence of various phytoconstituents in *Amaranthus blitum* L. leaves in noticeable quantities like flavonoids, alkaloids, saponins, lipids which are having many diverse medicinal benefits. The quantity and compounds found in TLC of flavonoids were in highest amount. The HPTLC fingerprint analysis of methanolic extract of leaves gives idea about the authentication of the extract by providing semiquantitative information about the phytoconstituents present in drug. Thus, the findings from this study will be helpful to check quality and purity of *Amaranthus blitum* L. leaves.

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