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Pharmacognostical, phytochemical and physicochemical evaluation of *Amaranthus blitum* L. leaves from south Gujarat

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Abstract
The present investigation was carried out to evaluate various quality control parameters of *Amaranthus blitum* L. leaves. The morphological features included simple leaf, ovate - lanceolate shape, tapering base, entire margin, obtuse, retuse, mucronate and emarginate apex with pinnate reticulate venation. Microscopy of leaf showed presence of palisade parenchyma interrupted with peripheral bundle sheath, rosette calcium oxalate, collateral closed vascular bundles, uniseriate multicellular covering trichomes. Leaf constants like stomatal index, vein islet number were also evaluated. Preliminary physicochemial screening revealed the presence of alkaloids, glycosides, steroids, tannins and phenolics. Fluorescence analysis of leaf powder showed different fluorescence with various chemical and reagents. The physicochemical parameters like ash values, extractive values, moisture content, foaming index were evaluated to establish quality standards. TLC study of various extracts were performed in different solvent systems to have an idea about presence of phytocostituents. Since there are many closely related species of the *Amaranthus* genus are available, this work will help in proper pharmacognostical identification of the plant.

Keywords: *Amaranthus blitum*, macroscopy, microscopy, physicochemical analysis, TLC

1. Introduction
Amaranthus blitum L. [Synonym: *Amaranthus lividus* L.] [1], belonging to genus *Amaranthus* which is collectively known as amaranth is annual or more rarely short-lived perennial, monoecious or dioecious plants distributed worldwide in warm and humid regions [2,3,4]. The genus *Amaranthus* consists of around 70 species in the world, 17 of which are edible [5]. *Amaranthus blitum* L. is spreaded all over the world ranging from tropical to temperate areas like Japan and Western Europe. The cultivated type is probably originated from India, where it holds a position of an important green leaf vegetable [6]. The plant is occurred in rainy season and also in farms with other crops. It is also known as "Ukedi bhaji" and "Adbau Tandaljo" [7]. The leaves are claimed to be used in liver and kidney disorders, fever, hemorrhage, anemia. It has laxative property and also used to enlarge spleen in hepatic disorder [6, 8]. In present work, pharmacognostical, physicochemical and phytochemical characters of *Amaranthus blitum* L. were studied to explore authentic plant material for its identification and medicinal claims.

2. Materials and Methods
2.1 Collection and authentication of plant
Fresh leaves of the selected plants were collected from surrounding area of Valod, Tapi District, Gujarat, India during month of July 2015. The plant was authenticated by NISCAIR, New Delhi with the reference no. NISCAIR/RHMD/Consult/2015/2872/65 dated 13/08/2015. The leaves were carefully washed with water to remove adherent dust, dried in shade, coarsely powdered and stored in air tight containers for further study.

2.2 Macroscopy
The fresh leaves were taken and observed for macroscopic characters like size, shape, colour, odour, taste, surface venation [9, 10].

2.3 Microscopy
2.3.1 Qualitative microscopy
The qualitative microscopic study was carried out by taking thin free hand transverse sections of fresh leaf and petiole. The sections were cleared with chloral hydrate solution, stained with phloroglucinol and concentrated hydrochloric acid in ration of 1:1 and washed with water to wash away the excess stain.

The stained sections were mounted with glycerin on a clear slide and observed under compound microscope. The powder of dried leaves was also stained in same manner and observed under microscope and diagnostic characters were recorded [11].

2.3.2 Quantitative microscopy
The quantitative microscopy was carried out by evaluating leaf constants such as stomatal number, stomatal index, vein islet number, vein termination number and palisade ratio by using epidermal peels of the fresh leaf [12].

2.4 Preliminary Phytochemical screening
Phytochemical screening of different extracts petroleum ether, chloroform, ethyl acetate, ethanol, methanol, and aqueous prepared by cold maceration method were carried out by performing qualitative tests to detect presence or absence of various phytoconstituents [11].

2.5 Physicochemical analysis
The various physicochemical parameters such as extractive values, ash values, moisture content and foaming index were determined as per the official methods [13, 14].

2.6 Fluorescence analysis of leaf extracts and leaf powder
A small quantity (1 ml) of petroleum ether, chloroform, ethyl acetate, ethanol, methanol, and aqueous extracts were subjected to fluorescence analysis by observing the color differences in day light, short UV light (254 nm) and long UV light (365 nm).
A pinch of dried leaf powder was mixed with freshly prepared reagents and solvents followed by recording of color in day light, short UV light (254 nm) and long UV light (365 nm) [15, 16].

2.7 TLC study
All extracts were studied for thin layer chromatography using silica gel G as an adsorbent in various mobile phases. The developed plates were derivatized in iodine vapor and R_f value of each spot was calculated [17].

3. Results and Discussion
3.1 Macroscopy

Table 1: Macroscopic characters of fresh leaf of *A. blitum* L.

S. No.	Macroscopic character	Description
1	Leaf type	Simple
2	Arrangement	Alternate
3	Stipule	Exstipulate
4	Size	10 to 13 cm length; 5 to 6 cm width
5	Petiole	7.5 to 9.5 cm long , deeply grooved and slightly curved
6	Shape	Ovate –lanceolate
7	Color	Upper surface- dark green Lower surface- pale green
8	Odour	Characteristic
9	Taste	Bitter
10	Apex	emarginate/retuse /obtuse with pitted spine like structure
11	Base	Symmetric, Tapering
12	Margin	Entire
13	Venation	Pinnate reticulate, prominent on lower surface, lateral veins proceed towards leaf apex
14	Surface	Smooth

Fig 1: Macroscopic characteristics of *A. blitum* leaf

The leaf of *Amaranthus blitum* L. revealed the morphological characters as shown in Table 1 and Fig. 1. The leaf was simple, alternate, ovate - lanceolate shape, tapering base, entire margin, obtuse, retuse, mucronate and emarginate apex with pinnate reticulate venation. The leaf size was 10-13 cm X 5-6 cm, dark green above and pale in beneath with bitter taste and characteristic odour. The grooved petiole was found longer than leaf blade and slightly curved.

3.2 Microscopy
3.2.1 Qualitative Microscopy
Leaf
The transverse section passing through midrib of *Amaranthus blitum* L. leaf shown in Fig. 2. The leaf was dorsiventral in structure. The upper and lower epidermises were single layered comprising of straight wall, more or less rectangular shaped cells covered with thin cuticle. The palisade parenchyma were arranged in a single layer and interrupted with peripheral bundle sheath enclosing small vascular bundle which is also known as Kranz leaf anatomy.The spongy parenchyma in mesophyll was loosely arranged in 3-4 layers. Rossette shaped calcium oxalate were also present in mesophyll. The number of covering trichomes were present. Trichomes were multicellular, uniseriate with bulbous or acute apex. Some trichomes also showed collapsed cells. The midrib showed 2-3 layered thick walled collenchyma below upper epidermis and above lower epidermis. Around 13 small collateral closed vascular bundles were arranged in a cordate shape in midrib. The ground tissue was comprised of closely packed thin walled parenchymatous tissue with rosette shaped calcium oxalate.

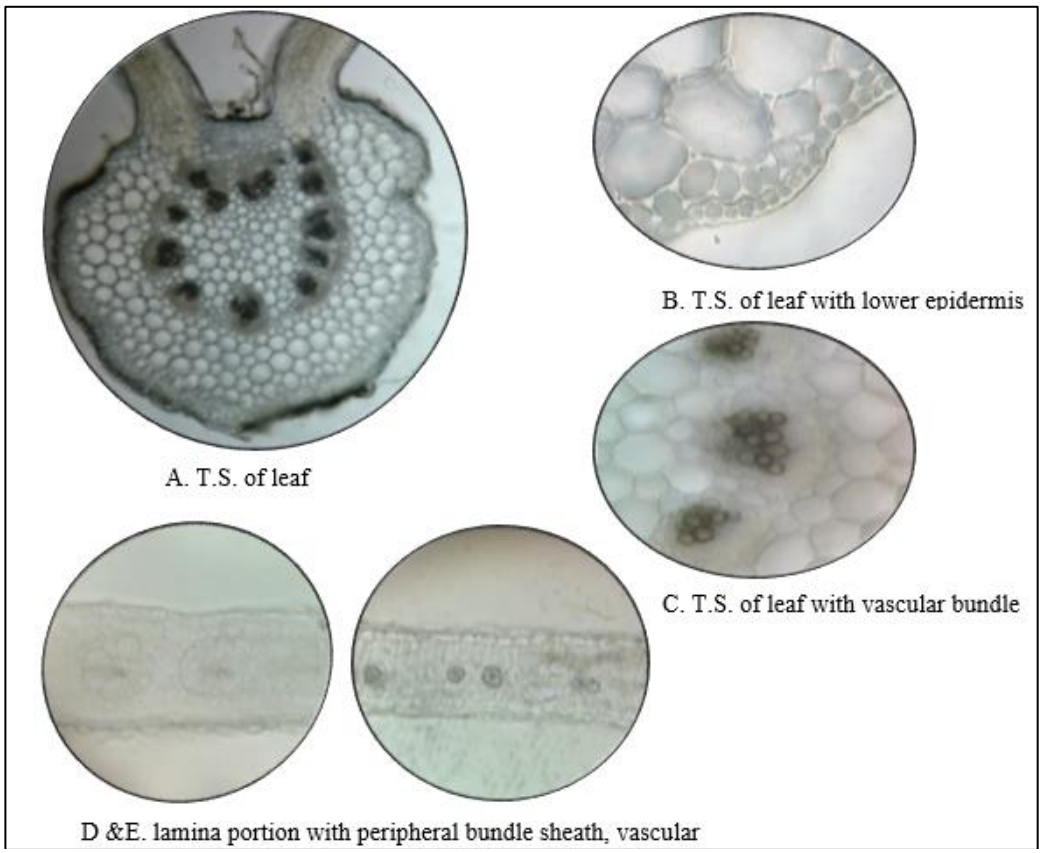


Fig 2: Microscopic characters of *A. blitum* L. leaf T.S.

Petiole

The transverse section of *Amaranthus blitum* L. leaf petiole is shown in Fig. 3 reveals cordate shape. The epidermis was single layered parenchymatous cells covered with cuticle. Epidermis was followed by 5-7 layers of hypodermis comprising of angular collenchyma. The ground tissue was made up of parenchyma and 10 -13 closed collateral vascular bundles arranged in a crescent shape. Scarce number of trichomes were present which are similar in characteristics like that of leaf trichomes

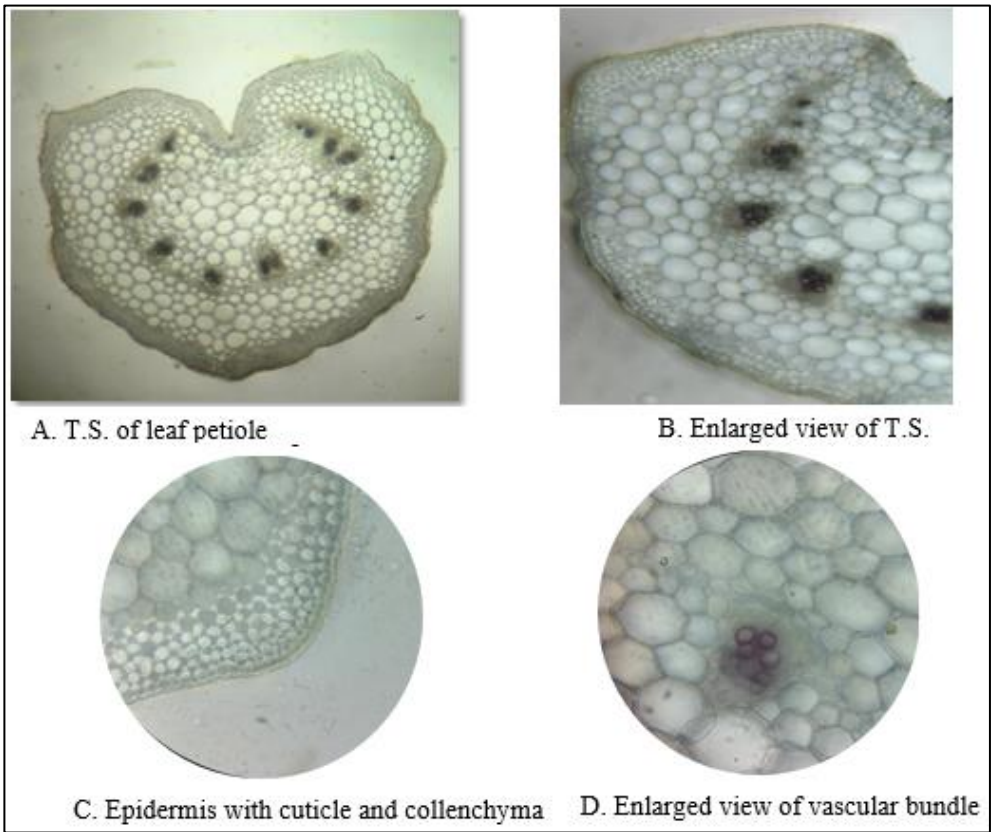


Fig 3: Microscopic characters of leaf petiole T.S

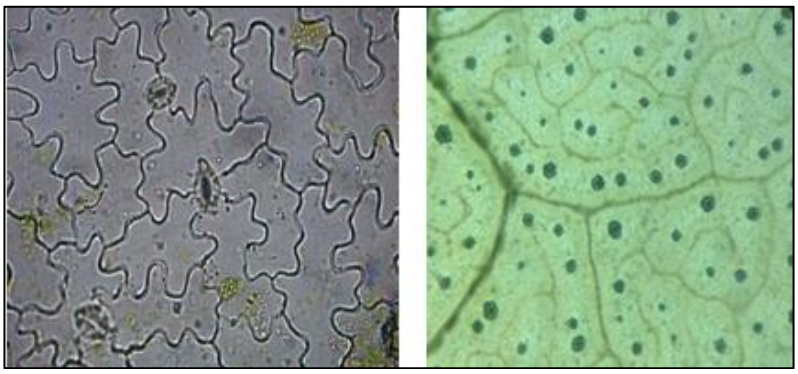
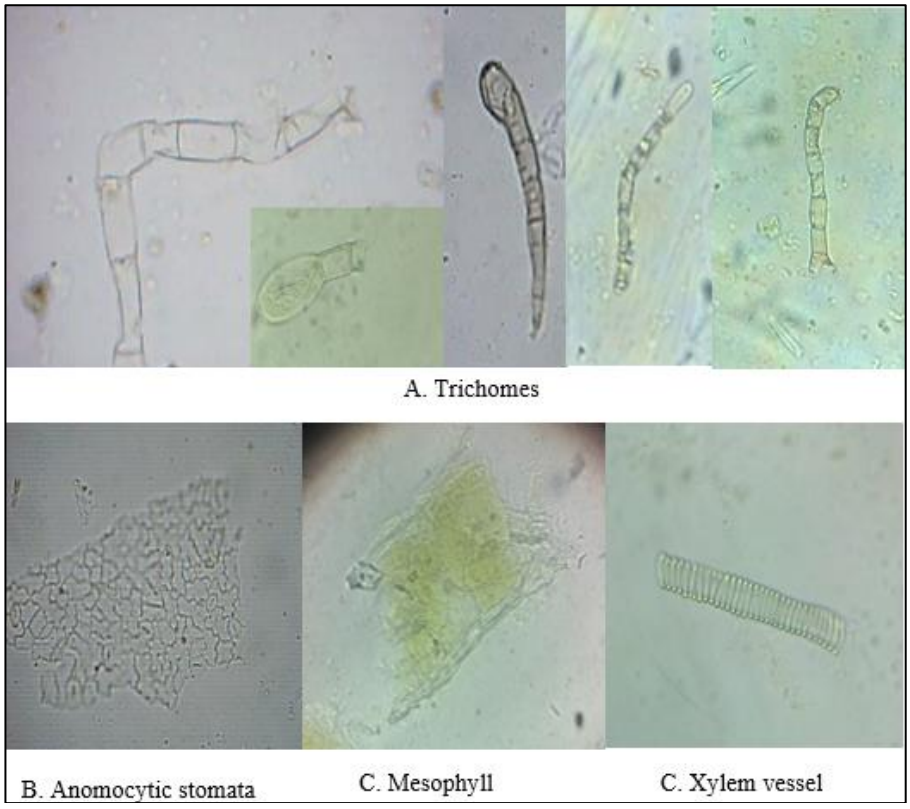


Fig 4: Anomomcytic stomate

Fig 5: Venation pattern

Powder study

The powder characteristics of *Amaranthus blitum* L. leaf are shown in Fig. 6. The specific characters determined in microscopy were trichomes, anomocytic stomata, spiral xylem vessels, mesophyll, and rosette shaped calcium oxalate and starch grains.



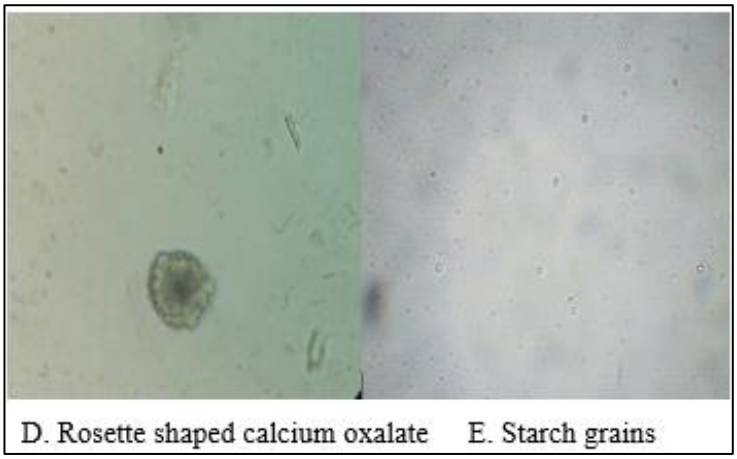


Fig 6: Powder characteristics of *A. blitum* leaf

3.2.2 Quantitative microscopy

The parameters of quantitative microscopy such as stomatal number, stomatal index, vein islet number, vein termination number and palisade ratio were evaluated and results were tabulated as shown in Table 2.

3.3 Preliminary Phytochemical screening

Preliminary phytochemical screening of *Amaranthus blitum* L. leaves by performing various qualitative tests showed presence or absence of various phytoconstituents in different extracts. The detailed result is given in Table 3.

Table 2: Quantitative microscopy of *A. blitum* L. leaf

S. No	Leaf constants		Results (mm^2)
1	Stomatal number	Lower	103 ± 2.86356
		Upper	66 ± 1.46287
2	Stomatal index (%)	Lower	20.6 ± 1.46969
		Upper	15.8 ± 1.24097
3	Vein islet number		11 ± 0.83666
4	Vein termination number		9 ±1.02956
5	Palisade ratio		4.95 ± 0.67268

Table 3: Preliminary phytochemical screening of *A. blitum* L. leaf

Plant constituents	Tests performed	Pet.ether ext.	Chloroform ext.	Ethyl acetate ext.	Methanol ext.	Ethanol ext.	Aqueous ext.
Protein & amino acid	Biuret test	-	+	-	-	+	+
	Million's test	-	+	+	+	+	+
	Xanthoprotein test	-	-	-	-	-	-
	Ninhydrin test	-	+	+	+	+	+
Carbohydrates	Molisch's test	-	+	-	+	+	+
	Fehling's test	-	+	-	-	+	+
	Benedict's test	-	-	-	+	+	+
Fixed oil and fats	Stain test	-	+	+	+	+	+
Alkaloids	Dragendroff's test	-	-	-	+	+	+
	Mayer's test	-	-	-	+	+	+
	Wagner's test	-	-	-	+	+	+
	Hager's test	-	+	-	+	+	+
Cardiac glycosides	Legal's test	-	-	-	-	-	-
	Kellerkilliani test	-	-	-	-	+	+
	Libermann test	-	+	+	-	_	-
Anthraquinones	Born trager's test	-	-	-	-	_	-
	Modified borntrager's test	-	+	-	+	+	+
Cynogenetic	Mercuric nitrate	-	-	-	-	-	-
Flavonoids	Shinoda test	powder (+)					
Saponin	Foam test	powder (+)					
Steroids	Salkowski test	+	+	+	-	-	-
	Libermann Burchard test	+	+	+	-	-	-
	Libermann	-	+	+	-	-	-
Volatile oil	Stain test	+	-	-	-	-	-
Phenolics and Tannins	5% Fecl3	-	+	-	+	+	+
	Lead acetate	-	-	-	+	+	+
	Acetic acid	-	-	-	+	+	+
	Dilute Iodine	-	-	-	+	+	+
	Dilute KMnO4	-	-	-	+	+	+

["+"=Present, "-"= Absent]

3.4 Fluorescence analysis

The extracts prepared by cold maceration and treated powdered drug of leaf was various observed in day light, short UV light (254 nm), long UV light (365 nm) and results were tabulated as shown in Table 4 and 5 respectively.

Table 4: Fluorescence analysis of extracts *A. blitum* L. leaf

S. No	Extract	Day light	UV short	UV long
1	Petroleum Ether (60°-80°)	Light green	Light green	Orange
2	Chloroform	Brownish green	Brown	Orange
3	Ethyl acetate	Dark green	Light brown	Orange
4	Methanol	Dark green	Brown	Orange
5	Ethanol	Yellowish green	Light green	Fluo.Orange
6	Water	Brown	Green	Fluo.Green

Table 5: Fluorescence analysis of Powdered *A. blitum* L leaf

S. No	Treatments	Day light	UV short	UV long
1	Powder(as such)	green	green	light green
2	Distilled water	brown	green	green
3	Picric acid	yellowish green	light green	fluorescent green
4	Glacial acetic acid	light brown	green	orange
5	1 N HCl	light brown	light green	fluorescent green
6	1 N H_2SO_4	brown	green	fluorescent green
7	Conc. HNO_3	brown	light green	light green
8	5% $FeCl_3$	brown	green	green
9	5% Iodine	brown	green	green
10	Ammonia solution	light green	green	fluorescent green
11	1N NaOH (Aqueous)	brownish green	dark green	light green
12	1 N NaOH (Alcoholic)	green	green	orange
13	HNO_3+ Ammonia	brownish green	light green	fluorescent green
14	Methanol	green	light green	orange
15	Toluene	green	green	orange
16	Benzene	light green	green	orange
17	Lead acetate	white	white	greenish white
18	Acetic anhydride	light green	light green	orange
19	Pet. ether (60°-80°)	light green	light green	orange
20	chloroform	brownish green	light green	orange

3.5 Physicochemical analysis

The powdered drug was studied for it's physicochemical parameters like ash values, extractive values, moisture content etc. and results were calculated (Table 6).

Table 6: Physicochemical analysis of *A. blitum* L. leaf

S. No.	Parameter		Values (%w/w) Mean ± SEM
1	Ash values	Total ash	13.66± 0.600
		Acid insoluble ash	3.06 ± 0.366
		Water soluble ash	3.00 ± 0.06
		Sulphated ash	22.00 ±0.333
2	Extractive values	Petroleum Ether (60°-80°) soluble	1.16 ± 0.166
		Chloroform soluble	2.16 ± 0.441
		Ethyl acetate soluble	1.66 ± 0.166
		Methanol soluble	10.46 ± 0.290
		Ethanol soluble	10.66 ± 0.666
		Water soluble	24.43 ± 0.983
3	Moisture content	on dry basis	11.333 ±0.166
		on fresh basis	16.167 ± 0.166
4	Foaming index	Less than 100	

3.6 TLC profile

The all extracts prepared by cold maceration were subjected to thin layer chromatographic studies by using different mobile phases. After derivatization with iodine vapours, Rf values were calculated of respective spots. The detailed results are shown in Table 7

Table 7: TLC study of *A. blitum* L. leaf

Extract	Solvent system	Ratio	No. of spots	R_f values
petroleum ether extract	n-butanol:acetic acid:water:formic acid	3:2:5:0.1	1	0.73
	petroleum ether:chloroform	0.5:9.5	5	0.07, 0.15, 0.39, 0.76, 0.94
	butanol:methanol	9.5:0.5	2	0.40, 0.59
chloroform extract	n-buatanol:acetic acid:water	3.0:2.0:5.	2	0.83, 0.91
	ethyl acetate :methanol:water	7.5:1.25:0.5	4	0.38, 0.67, 0.83, 0.97
Ethyl acetate extarct	n-butanol:acetic acid:water:formic acid	3:2:5:0.1	2	0.62, 0.86
	chloroform:methanol	9.5:0.5	7	0.04, 0.08, 0.2, 0.26, 0.46, 0.62, 0.93
Methanolic extarct	n-butanol:acetic acid:water:formic acid	4:2:4:0.1	3	0.63, 0.82, 0.91
	chloroform:ethyl acetate:methanol:water	5:2.6:1.3:0.3	6	0.10, 0.21, 0.52, 0.65, 0.80, 0.95
Ethanolic extract	n-butanol:acetic acid:water	04:02:04	3	0.54, 0.88, 0.98
	chloroform:ethyl acetate:methanol:water	5:2.6:1.3:0.3	6	0.01, 0.12, 0.21, 0.75, 0.83, 0.96
	n-butanol:acetic acid:water:formic acid	4:2:4:0.1	2	0.2, 0.62
	chlorogorm:methanol	9.3:0.7	3	0.03, 0.85,0.94
	hexane:ethyl acetate	08:02	6	0.09, 0.16, 0.31, 0.37, 0.55, 0.64
Distilled water extract	n-butanol:acetic acid:water	04:02:04	3	0.58, 0.62, 0.91
	acetic acid:hydrochloric acid:water	10:01:03	1	0.02

4. Discussion

The macroscopic characters of the fresh leaves of *Amaranthus blitum* L. were studied as per the standard description of terms. The observations were recorded in Table 1 following simple leaf; ovate-lanceolate shape; emarginate, retuse, obtuse apex; entire margin; tapering base withlong grooved petiole. Fig. 2 reveals the microscopic characters of leaf T.S. passing through midrib showed dorsiventral leaf with single layered epidermis; single layer of palisade parenchyma interrupted with peripheral bundle sheath enclosing small vascular bundle and rosette calcium oxalates. Multicellular uniseriate covering trichomes were present on both upper and lower epidermis. The transverse section of leaf petiole having cordate shape was covered with single layered epidermis, hypodermis made up of angular collenchymas, parenchymatous ground tissue and 10-13 closed collateral vascular bundles. The microscopic study of leaf powder showed the multicellular, uniseriate covering trichomes with bulbous or acute apex; anomocytic stomata; spiral xylem vessels; mesophyll with peripheral bundle sheath; rosette calcium oxalate and simple starch grains. The anomocytic stomata on lower surface and venation pattern are shown in Fig.4 and 5 respectively. Table 2 revealed the values for leaf constants like stomatal number, stomatal index, vein islet number, vein termination number and palisade ratio. These values can be used for standardization as well as identification of closely related species of plant [18]. However, the variations in results could be found in plants with different geographical conditions [19]. The qualitative preliminary phytochemical screening of extracts reveled presence of alkaloids in methanol and aqueous extracts; glycosides in chloroform, methanol, ethanol and aqueous extracts; flavonoids and saponin in dry powder; tannins and phenolic compounds in methanol, ethanol and aqueous extracts; steroids were present in petroleum ether, chloroform and ethyl extracts; protein , carbohydrate and fats were found in all extracts except petroleum ether extract while volatile oil was found to be present in petroleum ether extract. The fluorescence analysis is also helpful in identification of authentic samples and recognition of adulterants [20]. The powdered leaf drug showed different colours with fluorescence when treated with various chemicals and reagents. The determination of ash values measures the amount of sand, siliceous earth and other impurities. The total ash, acid insoluble ash, water soluble ash and sulphated ash were 13.66± 0.600, 3.06 ± 0.366, 3.00 ± 0.06 and 22.00 ±0.333 respectively. The maximum extractive value was found in water (24.43 ± 0.983). Methanol and ethanol also gave significant values 10.46 ± 0.290 and 10.66 ± 0.666 respectively. The moisture content was 11.333 ±0.166 on dry basis and 16.167 ± 0.166 on wet basis in optimum range. The higher water content leads to decomposition of the drugs due to microbial attack or chemical changes [21]. The foaming index was less than 100. Thin layer chromatogrphic studies of all extracts revealed the various numbers of spots in different mobile phases revealing wide range of phytochemicals present in drug. The ethyl acetate extract showed maximum number of spots (7) in chloroform: methanol (9.5:0.5). Methanolic and ethanolic extracts showed 6 spots in chloroform: ethyl acetate: methanol: water (5:2.6:1.3:0.3). Ethanolic extract also showed 6 spots in hexane: ethyl acetate (8:2).

5. Conclusion

The present study was focused on evaluation of various parameters like macroscopy, qualitative and quantitative microscopy, preliminary phytochemical investigation, physicochemical standards and TLC profile of *Amaranthus blitum* L. leaves which will help in identification and detection of adulteration of this plant. These findings will also be helpful in distinguishing the closely related species of same genus and family.

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