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COMPARATIVE STUDIES ON *AMARANTHUS VIRIDUS* AND *AMARANTHUS SPINOSUS*

S. Swarnakumari [*1], S. Mohan [2], M. Sasikala [3] and T. Umapoorani [4]

Department of Pharmacognosy [1], Department of Pharmaceutics [2], Department of Pharmaceutical Chemistry [3], Department of Pharmacognosy [4], Karpagam College of Pharmacy, Coimbatore - 641032, Tamil Nadu, India.

Keywords:

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Correspondence to Author:
S. Swarnakumari

Department of Pharmacognosy, Karpagam College of Pharmacy, Coimbatore - 641032, Tamil Nadu, India.

E-mail: swarnapremanand@gmail.com

ABSTRACT: Objective: To correlate the pharmacognostic appearance and physiochemical specification of the leaves of *Amaranthus viridus* and *Amaranthus spinosus*. **Methods:** Pharmacognostical features were scrutinized using fresh and dried powder, and physicochemical assessment was done by WHO guidelines. The phytochemical screenings were adopted for disclosing the existence of phytoconstituents in extracts. **Results:** The organoleptic features have been recorded for the fresh and powder material of *Amaranthus viridus* and *Amaranthus spinosus*. Microscopical examination reveals the arrangement of stomata, trichomes, and parenchymatous cells in both *Amaranthus* species. Phytochemical screening with discrete extracts affirms the existence of different phytoconstituents like alkaloids, carbohydrates, glycosides, tannins, saponins, flavonoids, protein, and amino acids. Identification of Physicochemical characters is useful in distinguishing the powdered drug material. Based on Phytochemical screening results, alkaloid content was determined by spectrophotometric methods and reported more amount in *Amaranthus viridis* when compared to *Amaranthus spinosus*. **Conclusion:** The current research favours in an attempt to expand the data with respect to its standardization, identification and exploration of medicine.

INTRODUCTION: From the traditional ages, herbal drugs are usually made using mixtures of constituents from various plant drugs [1]. Herbs, from starting raw material to finished products, are considered Herbal medicine [2]. Consistent qualities of herbal medicines are purely based on the quality of starting material. Since raw materials influences over the quality, efficacy, and safety of finished herbal preparations, standardization of raw materials is required [3]. The standardization of a crude drug involves pharmacognostic and Phyto-chemical studies.

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Amaranthus viridis (Fam: *Amaranthaceae*) is an annual herb known as slender amaranth. It is an edible herb in southern parts of India and also used in ayurvedic formulation due it's numerous pharmacological efficiencies [4]. *Amaranthus spinosus*, commonly known as thorny amaranth, also belongs to the family *Amaranthaceae* native to America. It is used as folk medicine and is traditionally reported to possess more activities [5].

MATERIAL AND METHODS:

Plant Material: The leaves of *A. viridis* and *A. spinosus* were collected in Coimbatore District, Tamil Nadu, India, and authenticated by Dr. C. Murugan, Scientist 'D' & Head of office, T.N.A.U, Coimbatore, Tamil Nadu, India. Authentication number BSI/SRC/5/23/2018/Tech-2897. The leaves of each plant were segregated from the whole plant, dried in the shade with ambient temperature, grounded and stored well.

Morphological Study of the Plants: The organo-leptic characters of the fresh leaves and powder material of *Amaranthus viridus* and *Amaranthus spinosus* were ascertained [6].

Microscopical Study of the Plants: Microscopical characters for both the plants *Amaranthus viridis* and *Amaranthus spinosus* were scrutinized [7].

Histological Characters of the Plant: Fresh leaves of *Amaranthus viridis* and *Amaranthus spinosus* were collected and immediately made into transverse section as per technique [8]. Chlorophyll pigment is removed using chloral hydrate and strained using Phloroglucinol and con HCl [9].

Powder Microscopy: Fresh leaves of *Amaranthus viridis* and *Amaranthus spinosus* were gathered, and then powder was identified for the existence of discrete cells [9].

Leaf Constant: Leaf constants present in both the plant leaves were determined [9].

Physiochemical Evaluation: The crude powdered drugs were subjected to various physiochemical investigation.

Total Ash: 2 g of powdered drugs of leaves of *Amaranthus viridis* and *Amaranthus spinosus* were tarred in silica crucible at 450 °C. The residues were cooled in a suitable desiccator. The total ash was determined [10].

Water- Soluble Ash: In the crucible, boil ash with H_2O for 5 min using ashless filter paper; the insoluble contents are collected, ignited in a silica crucible, and weighed after cooling. On subtraction of insoluble ash weight from total ash, the water-soluble content is calculated [10].

Acid-Insoluble Ash: 25 ml of Dilute Hydrochloric acid is mixed with total ash and boiled for 5 min. On filtering the content, acid insoluble matter was collected and also neutralized by washing with hot water. Dried filter paper along with insoluble matter was incinerated in silica crucible and cooled in desiccators [10].

Determination of Extractive Value: Based on Extractive value of medicinal plants, the quantity of active constituents can be determined. The solubility of phytochemicals is variable, and so different solvents are employed from polar to non-polar. 5 g of an accurately weighed powdered drug is extracted with alcohol (90%) in stopper flask for 6 h with frequent shaking. Later for about 18 h, the content is extracted on rotary shaker. Filtered and the filtrate is evaporated. The alcohol (90%) soluble extractive is figured. The same procedure was repeated with disparate solvents like chloroform, Benzene, Ethanol, Ethyl acetate and water. The above procedures have been conducted for *Amaranthus spinosus* and also for *Amaranthus viridus* and their extractive values were determined.

Preliminary Phytochemical Evaluation: To detect the existence of phytoconstituents, various solvent extracts of *Amaranthus viridis* and *Amaranthus spinosus* were *employed* [10, 15].

Determination of Total Alkaloids: Weighed powdered drug is mixed with 200 ml 10% acetic acid in ethanol and allowed to stand for 4 h. Filter and concentrate the extract to one-fourth of the volumes. Add drops of concentrated ammonium hydroxide till precipitation completes. Allow the precipitate to settle and wash with dilute ammonium hydroxide followed by filtration. The dried residue is weighed, and alkaloidal content is determined [16].

Alkaloids (%) = (weight of the extract)/(weight of the crude drug) × 100

Spectrophotometric Determination of Total Alkaloids:

Preparation of Solutions: 69.8 mg bromocresol green heated with 3 ml of 2N NaOH and 5 ml of distilled water for complete dissolution and further makeup to 1000 ml. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2 M sodium phosphate (71.6 g Na_2HPO_4 in 1 L distilled water) to 4.7 with 0.2 M citric acid. Atropine standard solution was made by dissolving 1mg pure atropine (Sigma Chemical, Bangalore) in 10 ml distilled water.

Preparation of Standard Curve: Accurately measure aliquots (0.4, 0.6, 0.8, 1, and 1.2 ml) of caffeine standard solution and transfer each to different separating funnels. Then 5 ml of (pH 4.7) phosphate buffers and 5 ml of BCG solution were mixed and shaken with 1, 2, 3, and 4 ml of chloroform, respectively.

The volumes of collected extract were adjusted with chloroform for 10 ml. blank performed without Atropine and absorbance measured.

Separation of Alkaloid: By hot continuous (Soxhlet) extraction method, grounded plant materials were extracted with methanol for a day. The filtered extract was placed in a rotary evaporator under vacuum at 45 °C for complete dryness. To one section of residue 2N HCL is added and filtered.

Transfer 1 ml of this solution to the separating funnel and wash with 10 ml of chloroform (3 times). Adjust the pH of the solution to neutral with NaOH.

To the neutral solution, 5 ml of BCG solution and 5 ml of phosphate buffer were added. Once extract this mixture with 4 ml of chloroform by vigorous shaking. Collect the mixture in 10 ml volumetric flask and make up with chloroform. The absorbance of the complex in chloroform was measured at the spectrum of 470 nm in UV-Spectrophotometer (SHIMADZU UV-1800) against the blank prepared as above but without Atropine [17].

RESULTS AND DISCUSSION:

Morphological Study of the Plants: Morphological characters of *Amaranthus viridis* and *Amaranthus spinosus* were viewed and compared in table and **Fig. 1**.

TABLE 1:

Parameters	*Amaranthus viridis*	*Amaranthus spinosus*
Colour	Green	Dark green
Odour	Odorless	Odorless
Taste	Bitter	Bitter
Stem	Weak stem without spines	Erect stem with spines
Leaves	Ovate, hairless, 1-10 cm long stalk	Elliptic – lanceolate, hair.
Flower	Pale white to green	Greenish -white

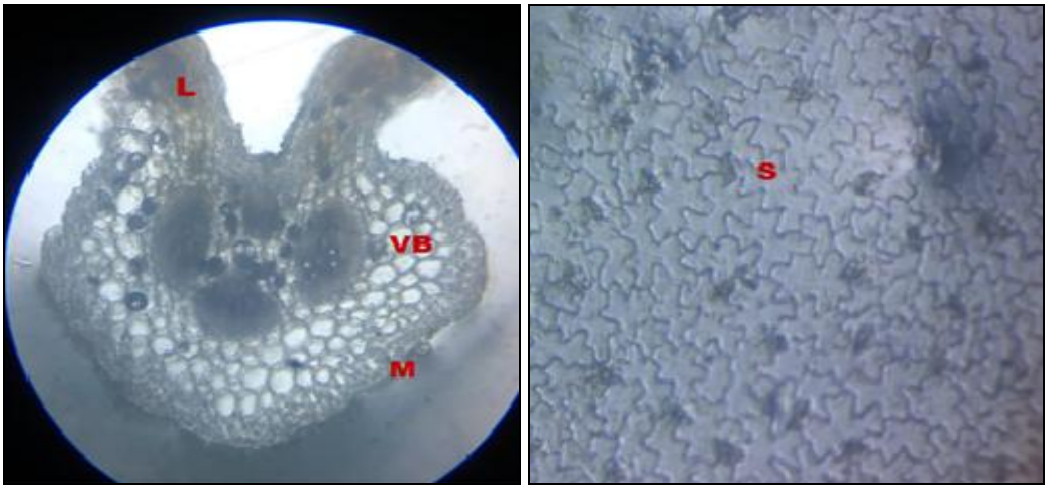
AERIAL PARTS OF *AMARANTHUS SPINOSUS* **AERIAL PARTS OF *AMARANTHUS VIRIDIS***

FIG.1: AERIAL PARTS OF *AMARANTHUS*VIRIDIS AND *AMARANTHUS*SPINOSUS

Histological Characters of the Plant: In the microscopical studies **Fig. 3** of *Amaranthus viridis*, the leaf was found to be dorsiventral. The midrib region shows the upper and lower epidermis with 3 vascular bundles in the center.

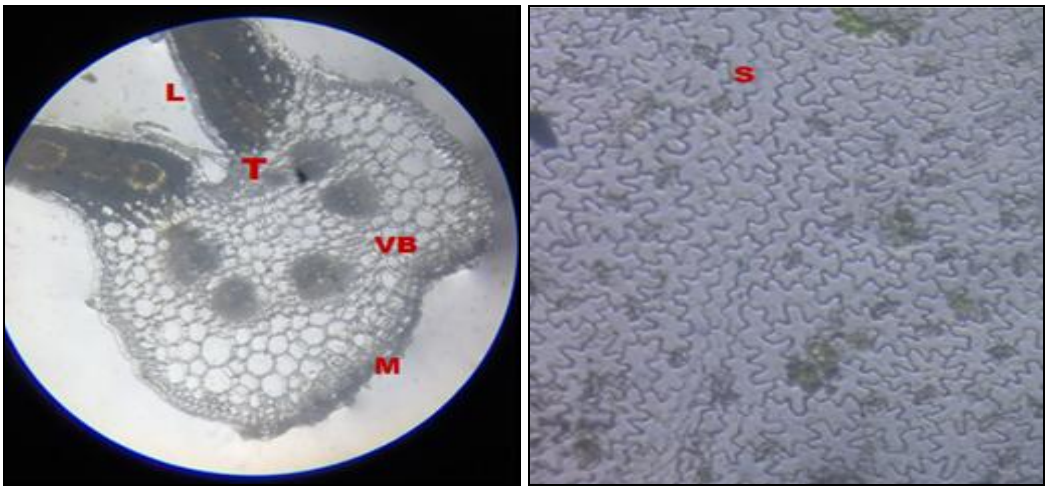
Palisade cells present in the upper part of the lamina, below that, followed by parenchyma cells. Anisocytic type of stomata is observed, and epidermal cells are found to be with wavy anticlinical walls. The leaf of *Amaranthus spinosus* was also found to be dorsiventral. The midrib region shows upper and lower epidermis, with 5 vascular bundles in the mesophyll region.

They were covering trichomes present on the upper surface. Palisade cells present in the upper part of the lamina, followed by parenchyma cells, and Anisocytic type of stomata is also noticed in **Fig. 4**.



LAMINA, VB VASCULAR BUNDLE, M- MIDRIB S-STOMATA

FIG. 2: TRANSVERSE SECTION AND STOMATAL ARRANGEMENT IN LEAVES OF *AMARANTHUS*VIRIDIS L-



L-LAMINA, VB VASCULAR BUNDLE, M- MIDRIB S-STOMATA

FIG. 3: TRANSVERSE SECTION AND STOMATAL ARRANGEMENT IN LEAVES OF *AMARANTHUS SPINOSUS*

Powder Microscopy:

TABLE 2: POWDER MICROSCOPY FOR THE LEAVES OF *AMARANTHUS VIRIDIS* AND *AMARANTHUS SPINOSUS*

Parameters	*Amaranthus viridis*	*Amaranthus spinosus*
Nature of the powder	Fine, smooth	Coarse
Colour	Green	Dark green
Odour	Odorless	Odorless
Taste	Bitter	Bitter
Shaken with water	No Frothing	No Frothing
Pressed in between two filter paper	No oil mark on the paper	No oil mark on the paper

TABLE 3: QUANTITATIVE MICROSCOPY FOR THE LEAVES OF *AMARANTHUS VIRIDIS* & *AMARANTHUS SPINOSUS*

Parameters	*Amaranthus viridis*	*Amaranthus spinosus*
Stomatal number	116	118
Stomatal index	22.3%	25%
Vein islet number	20.5/ sq.mm	46 / sq.mm
Veinlet termination	51.6/ sq.mm	97.8/ sq.mm

Quantitative Microscopy: Microscopical characters are quantitatively analyzed and reported in **Table 3**.

Physiochemical Evaluation: As per the procedure described, various ash values and extractive values were analyzed and reported.

Ash value of *Amaranthus viridisis* less when compared to *Amaranthus spinosus*. Methanolic Extractive values of *Amaranthus viridisis* 17% and *Amaranthus spinosusis* 5.2%

Preliminary Phytochemical Evaluation: Phytochemical screening of *Amaranthus viridis* & *Amaranthus spinosus* reported in **Table 4**.

TABLE 4: PRELIMINARY PHYTOCHEMICAL STUDIES FOR LEAF EXTRACT OF *AMARANTHUS VIRIDIS* AND *AMARANTHUS SPINOSUS*

S. no.	Phytoconstituents	*Amaranthus viridis*	*Amaranthus spinosus*
1	Alkaloid	+	+
2	Carbohydrate	+	+
3	Glycosides	+	+
4	Sterol	+	+
5	Saponins	-	-
6	Tannins	+	+
7	Proteins And Free Aminoacids	+	+
8	Flavonoids	+	+
9	Fixed Oils	-	-

+ indicates presence of the phytochemical, – indicates absence of the phytochemical

Determination of Alkaloids using Harborne Method: Based on the Harbone method, alkaloids were determined in both the plant's **Fig 4** and more amount of alkaloids were reported in *Amaranthus viridis* when compared to *Amaranthus spinosus* **Table 5.**

TABLE 5: DETERMINATION OF ALKALOIDS (USING HARBORNE METHOD)

S. no	Species	Parts	Alkaloids (%)
1	*Amaranthus viridis*	Leaves	26.7
2	*Amaranthus spinosus*	Leaves	23

AMARANTHUS VIRIDIS ***AMARANTHUS SPINOSUS***

FIG. 4: TOTAL ALKALOIDS IN *AMARANTHUS VIRIDIS* AND *AMARANTHUS SPINOSUS*

Spectrophotometric Determination of Total Alkaloids: The alkaloid content was examined in plant extract and expressed in terms of atropine equivalent as mg of extract (the standard curve equation: $y = 0.005 \times - 0.018$, $R^2 = 0.992$) in **Fig 5**. The amount of alkaloids in *Amaranthus viridis* was found to be more when compared with *Amaranthus spinosus* **Table 6.**

TABLE 6: SPECTROPHOTOMETRIC DETERMINATION OF TOTAL ALKALOIDS

S. no	Species	Parts	Total alkaloids mg AE/ 100 g
1.	*Amaranthus viridis*	Leaves	38.0 ± 0.18
2.	*Amaranthus spinosus*	Leaves	24.4 ± 0.12

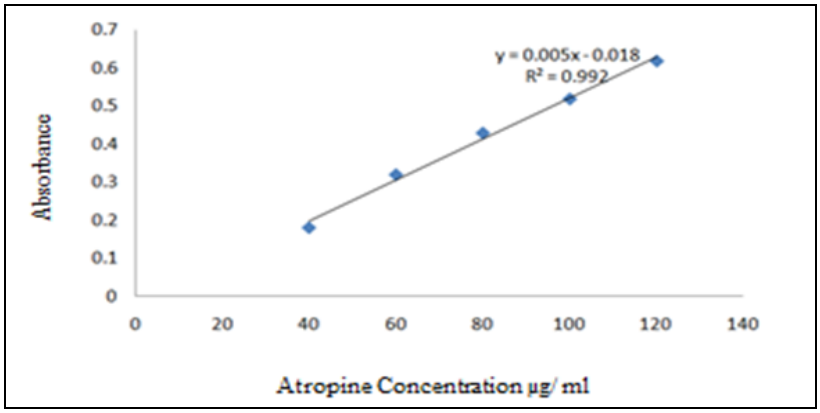


FIG. 5: STANDARD CALIBRATION CURVE FOR TOTAL ALKALOID CONTENT FOR STANDARD ATROPINE

CONCLUSION: The information obtained from the present study concludes that *Amaranthus viridis* is more effective and consists of more amount of alkaloids when compared to *Amaranthus spinosus.*

Since both the species of *Amaranthus* are edible and easily available, the phytochemicals can be isolated and utilized in drug development.

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CONTRIBUTION OF AUTHORS: All authors have contributed equally to carry out the work.

CONFLICTS OF INTERESTS: No conflicts of interest

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