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Pharmacognostical Standardization of *Amaranthus paniculatus* Seeds

Author(s): Bharti K. Parmar*, Dr. Navin Sheth

Herbal Drug Technology, Department of Pharmaceutical Sciences, Saurashtra University;, Rajkot, Gujarat, India.

Abstract

Pharmacognostical parameters for the seeds of *Amaranthus paniculatus* was studied with the aim of drawing the Pharmacognostical standards for this species. There is not systematic work reported according to WHO guideline, So objective for work in present study is, to prepare a Monograph of *Amaranthus paniculatus* seed according to WHO guideline. Macroscopical and microscopical characters of seed, powder characteristics studies, preliminary chemical test and thin layer chromotgraphic studies for *Amaranthus paniculatus* seed Different physicochemical parameters were evaluated. TLC and HPTLC were performed for identification of different Amino Acids. In its microscopic study, testa, endosperm, epidermis, cotyledon and radical were observed. In its powder characteristic study oil globules and starch were found. The qualitative analysis shows presence of glycoside, carbohydrate, protein and amino acids and fixed oils and absence of alkaloid and tannin was found. The TLC result shows presence of n-butyric acid, alanine, tyrosine, valine. The HPTLC results shows presence of ornithine, arginine, alanine, glutamic acid, tyrosine, n-butyric acid, methionine, leucine, isoleucine. The study includes pharmacognostical standardization of *Amaranthus paniculatus* Seeds first time.

Keywords: *Amaranthus paniculatus*, Macroscopy, Microscopy, cotyledon, Amino Acids.

Corresponding author: Ms. Bharti K. Parmar, Herbal Drug Technology, Department of Pharmaceutical Sciences, Saurashtra University; Rajkot, Gujarat, India. Contact-+918000315544; e-mail: bkparmar4@gmail.com

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Introduction

In India, medicinal plants have a good contribution to the development of ancient Indian medicines. One of the earliest treaties on Indian medicine, the Charak samhita (1000 B.C), records the use over 340 drugs of

vegetable origin. Most of these continue to be gathered from wild plants to meet the demand of the medical profession. Thus, despite the rich heritage of knowledge on the use of the plant drugs, little attention had been paid to grow them as field crops (Singh *et al.*, 2007).

Medicinal plants as a group comprise approximately 8000 species and account for around 50% of all the higher flowering plant species in India. Millions of rural households use medicinal plants in a self-help mode. Over one and a half million practitioners of the Indian system of medicine in the oral and codified streams use medicinal plants in preventive, promotive and curative application. Plants are the only economic source of a number of well established and important drugs. In addition, they are also the source of chemical intermediates needed for the production of some drugs. Search of chemical for discovering new drug is endless process. A huge contribution has come from merely 10% of the World's total biodiversity that has ever been explored. The proportion of drugs coming from natural resources is considerably high in case of anti-platelet drug (75%), anti-migraine (70%), anti-ulcer (47.6%), and anti-inflammatory (32.5%) (Verpoorte *et al.*, 1998).

In Ayurveda the seeds (beeja) are given a superior importance. Many drugs are used in the systems are seeds, they used to posses general qualities like smigdha (unctuous) due to fatty materials in it and many are used as balya (tonic) due to the high protein contents. Numerous properties besides these are also mentioned in Ayurveda. Many seeds are used in the food, like Green gram, Horse gram, Barley etc. uses, Besides these numbers of fixed oil are also used for their specific medicinal properties e.g. castor oil for its purgative properties, Chaulamoogra oil in laprocy, Karanj oil in leucoderma, scabies, herpes and other cutaneous skin disease. Jyotismati oil is used in beriberi, neem oil as antimicrobial and insect repellent. Some of their constituents like linoleic and linolenic acid – essential fatty acids- for lowering the cholesterol in the blood (Kokate *et al.*, 1990).

Amaranthus paniculatus (Amarnthaceae) is the world's most nitrous grain. In India, Amaranthus grain also called the Royal crop or RAMA's grain. The seeds of Rajgira are nutritious and its leaves are an important source of proteins and vitamins and minerals like Calcium, iron. It was reported that Amaranthus seeds are an alternative natural source of squalene. (Bhattacharjee Paramita *et*

al.2012) Amaranthus seeds has high energy value:407 kcal. (Rajyalakshmi *et al.*,1994.) The seeds contain 0.66% phospholipids. Major constituents of these phospholipids are: phosphoatidylethanolamine,

phosphatidylcholine and phosphatidylinositol; presence of small quantities of sphingomyelin and lysolecithin is also reported. The seed contain a high amount of protein 13.1% which is comparable to that of casein (Wealth of India 1986).

As seed have its medicinal value so study undertaken as 'pharmacognostical standardization of *Amaranthus paniculatus* Seeds'. This study is intended to establish, conventional pharmacognostical and modern pharmacognostical parameters of *Amaranthus paniculatus* Seeds. These will be use as diagnostic features in the identification, evaluation and monograph preparation of the *Amaranthus paniculatus* Seeds as per WHO guidelines.

Materials and Methods

Procurement of Seeds

Seeds of *Amaranthus paniculatus* were obtained from a local market in Rajkot City. Seeds were cleaned with water, then make dried, and powdered it. The plant material was taxonomically identified and authenticated by Dr. Sunita Gerg, NISCAIR, New Delhi, India, and the voucher specimen (SU/DPS/HERB/2013/57) was retained in the Department of pharmaceutical science, Rajkot for further references.

Chemicals and Instruments

Photomicroscope (OLYMPUS Pvt. Ltd., New Delhi; Model- CH 2OiBIMF) provided '3V-MICRO' video attachment eye piece device (Version8) with 10x, eye piece (12 mega pixel) with cells tracking function and 4x digital zoom camera was used. Solvents and reagents were procured from Loba Chemicals, Mumbai, India.

Preparation of Extracts:

Simple Soxhlet Extraction, ethanol extract is preaperd for *Amaranthus paniculatus* Seeds. By Cold maceration water extract prepared as, weigh about 5gm of powdered drug in a 250ml conical flask, and poured 100ml of distilled water. Set aside for 24hrs, shaking frequently. Filtered it. Evaporate to dryness on water bath, then cool and collected. Both Ethanol and water extract kept in desiccator for further use

Macroscopical Study

The macro- morphological features of the plant seeds were observed under magnifying lens and simple microscope (Tyler V., 1977).

Microscopical Study

Seeds were soft by boiling with water and microscopy studied by taking transverse sections (TS). The different parts of seeds like endosperm and cotyledon were studied

according to the methods of Brain and Turner, 1995. For the microscopical studies, cross sections were prepared and stained as per the procedure of K. R. Khandelwal, 2005. The different lens of photomicroscope as, OLYMPUS iNEA 5X, 10X/0.2; India, and 100X/1.25 oil India were used for capturing the photographs.

Powder Microscopy

A little quantity of seeds powder was taken onto a microscopic slide, 1-2 drops of 0.1% w/v phloroglucinol solution and a drop of concentrated hydrochloric acid were added and covered with a cover slip. The slide preparation was mounted in glycerol and examined under microscope. The presence of starch grain was detected by the formation of blue color on addition of 2-3 drops of 0.01 M iodine solution (Thitikornpong W *et al.*, 2011). The characteristic structures and cell components were observed and their photographs were taken using photomicrography.

Physicochemical Constant

The ash values were determined, to find out about the physiological state and level of extraneous matter. Moisture content of the powdered determined based on the loss of drying method.. Extractive values were determined according to the official methods prescribed in Ayurvedic Pharmacopoeia, 1985.

Phytochemical Investigation

The successive extractive values carry out as per the procedure of Harborne, 1984.

TLC Pattern for Extract of Seeds

The stationary phase and mobile phase set for Amino Acids as per the (Harborne, J.B., 1984)

Plate dimension- 10 x 10 cm.

Stationary phase- Silica gel G for TLC.

Sample preparation- 5mg water extract was dissolved in 5ml of acetone

Mobile phase- Various solvent systems have been tried for optimization of better resolution mainly using n-butanol- Aceitic acid-water.(4:1:1).

Visualization- Spraying by 0.1 % Ninhydrin reagent in acetone.

Treatment after spraying- Heated in oven at 105º c for 10 min.

HPTLC Pattern for Extract of Seeds

The stationary phase and mobile phase set for Amino Acids as per the (Harborne, J.B., 1984)

Plate dimension- 10 x 20 cm.

Stationary phase- Silica gel G for TLC.

Sample preparation- 5mg ethanol extract was dissolved in 5ml of ethanol.

Mobile phase- Various solvent systems have been tried for optimization of better resolution mainly using n-butanol- Aceitic acid-water.(4:1:1).

Visualization- Spraying by 0.1 % Ninhydrin reagent in acetone.

Treatment after spraying- Heated in oven at 105º c for 10 min.

Results and Discussion

Macroscopic Study

Seeds of *Amaranthus paniculatus* are obvoid to ellipsoid Compressed Around 1mm long, whitish to yellowish or blackish in color.

Fig.1: Seeds of *Amaranthus paniculatus*.

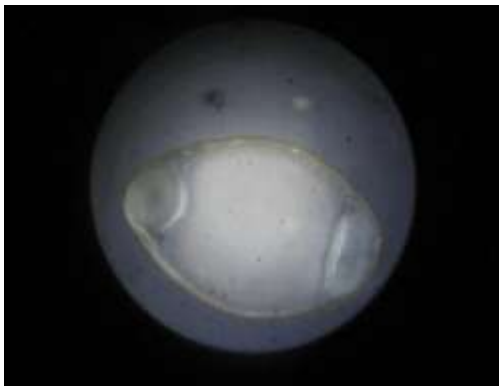


Fig. 2: TS of Seed

Fig. 3: Endosperm of Seed

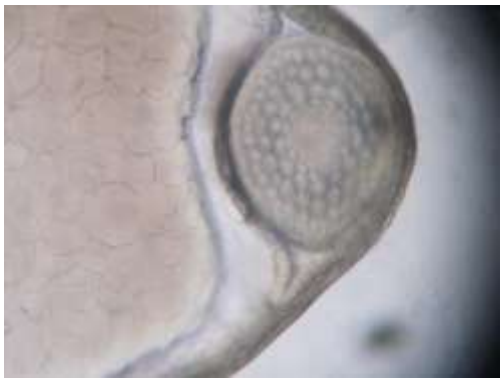


Fig. 4: Radicle of Seed

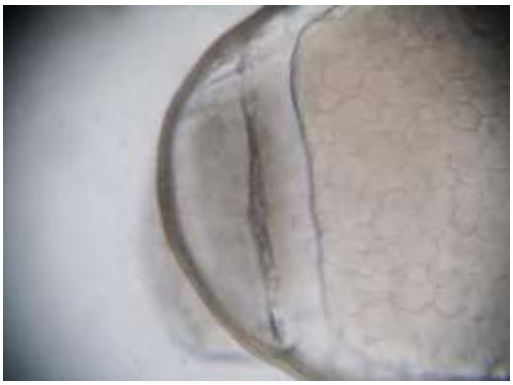


Fig. 5: Cotyledon of Seed

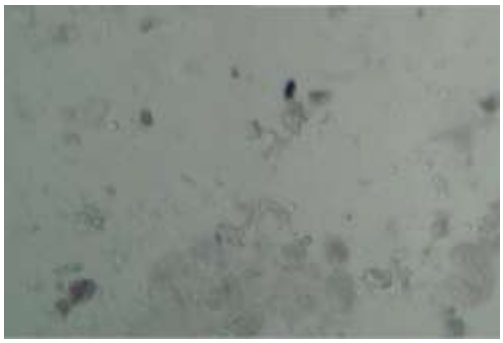
Microscopic Study

Fig. 2 -5 shows the Transverse section of Seeds shows the presence of upper epidermis, testa, endosperm, radicle, cotyledon, and lower epidermis.

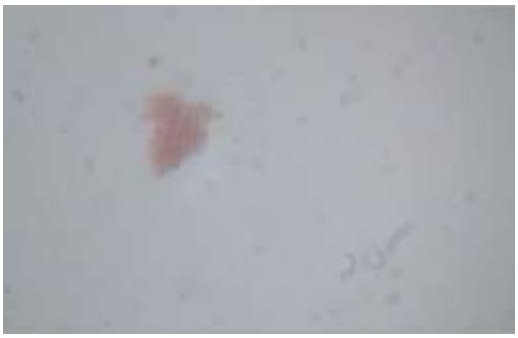
Powder Characteristics

The powder was white in color, on microscopically examination;

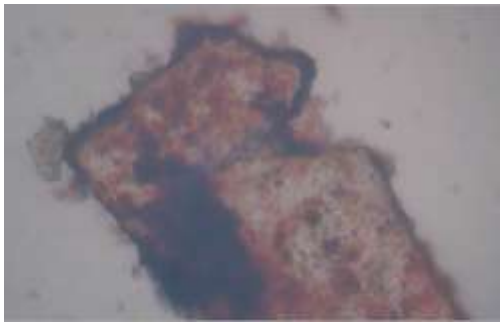
The powder shows oils globules (Fig. 6a) of fixed oils, cells of radicles (Fig. 6b), lignified tissues of paranchymatous cells (Fig. 6c) and simple starch grains (Fig. 6d).



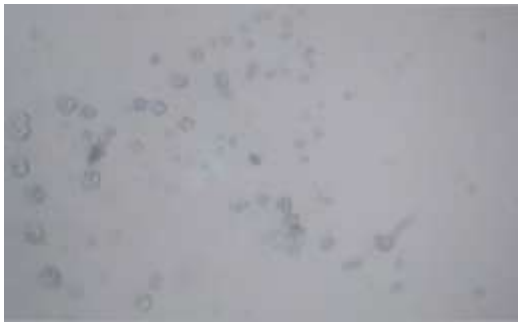
a. Oil Globules



b. Cell of Radicles



c. Parenchymatous Layer



d. Starch Grains

Fig. 6: Powder characteristic of *Amaranthus paniculatus* seeds

Physicochemical Study

Physical constant as ash value of the drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Extractive values are useful for the determination of exhausted or adulterated drugs. The moisture content seems to be lower than necessary to support the growth of microbes to bring any change in the composition of the drugs. (Table 1).

Phytochemical Investigation

Revealed the presence of primary and secondary metabolites as oils, glycosides, flavonoids, Phyto-steroids, amino acids and Fixed oils.

Table 1: Physicochemical Study of *Amaranthus paniculatus* Seeds

Parameter	%w/w
Total Ash Value	2.00
Acid - insoluble Ash Values	1.25
Water – soluble Ash Value	1.75
Ethanol (95%) Extractive Values	5.6
Water Soluble Extractive Values	10.4
Moisture content	1.5

TLC Fingerprint Profile

Thin layer chromatography of the water extracts was carried out. From TLC study we investigated there are n-Butyric acid, Alanine, Tyrosine, and Valine is present in extract of

Amaranthus paniculatus. The R_f were recorded in Table 2.

Table 2: TLC Fingerprint Profile of *Amaranthus paniculatus* Seeds

Amino Acids	Rf Value	
	A. paniculatus	**Standard**
n-butyric acid	0.48	0.48
Alanine	0.35	0.38
Isoleucine	0.53	0.66
Nor leucine	0.54	0.61
Tyrosine	0.51	0.73
Valine	0.58	-

HPTLC Fingerprint Profile

Chromatographic Development

The plate was developed with n-butanol-acetic acid-water (4:1:1) as mobile phase in Twin Trough Chamber with lid. Solvent front position kept 8cm, and then developed plates were dried in oven at 100 C for 5 minutes and subjected to scanning.

Chromatogram Evaluation and Estimation

Derivatization of plate carried out by spraying 0.1% Ninhydrin reagent in Acetone followed by heating at 110 C for 10 minutes. The CAMAG TLC Scanner was used for chromatogram evaluation. Plate was scanned at 254 nm and 366 nm (Fig. 2, 3 and 4)

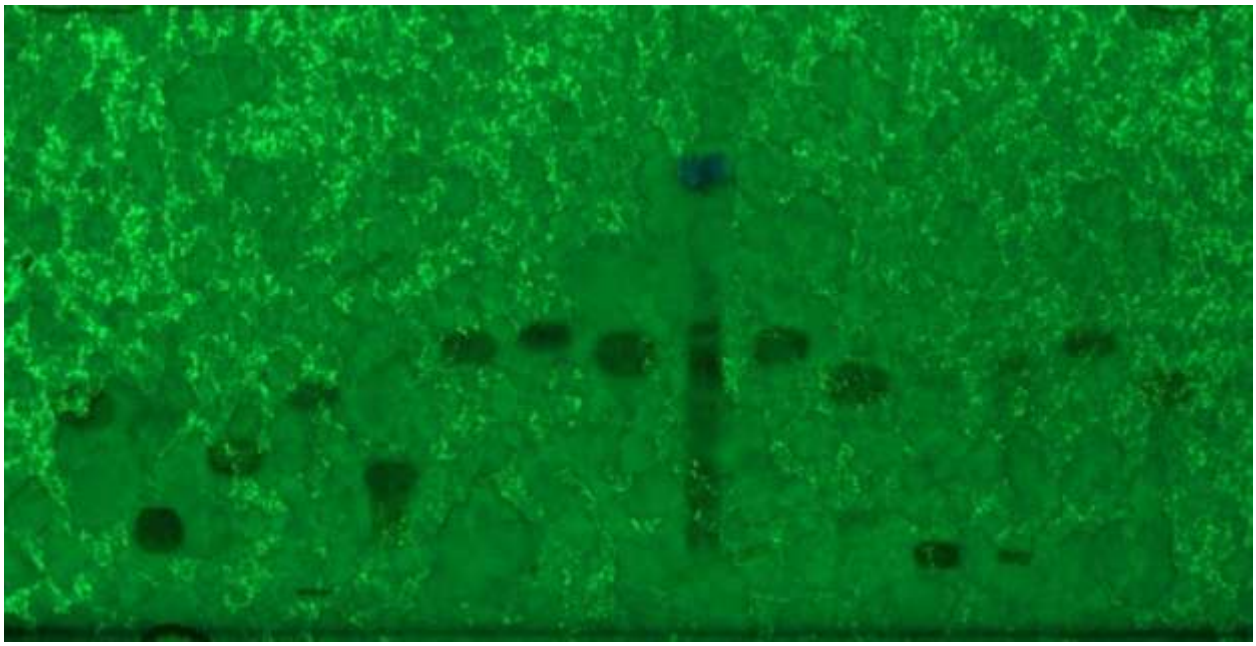


Fig. 2: HPTLC Plate of Std. Amino Acids and Seed's Ethanolic Extract Under 254nm

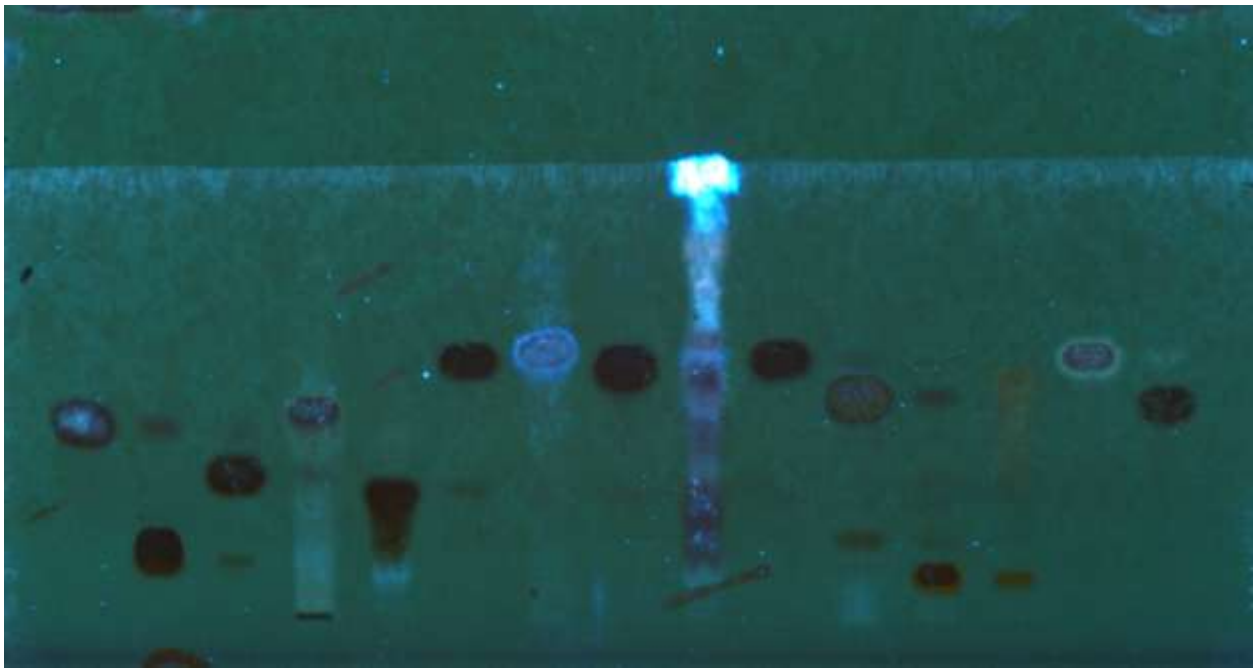


Fig. 3: HPTLC Plate of Std. Amino Acids and Seed's Ethanolic Extract Under 366nm

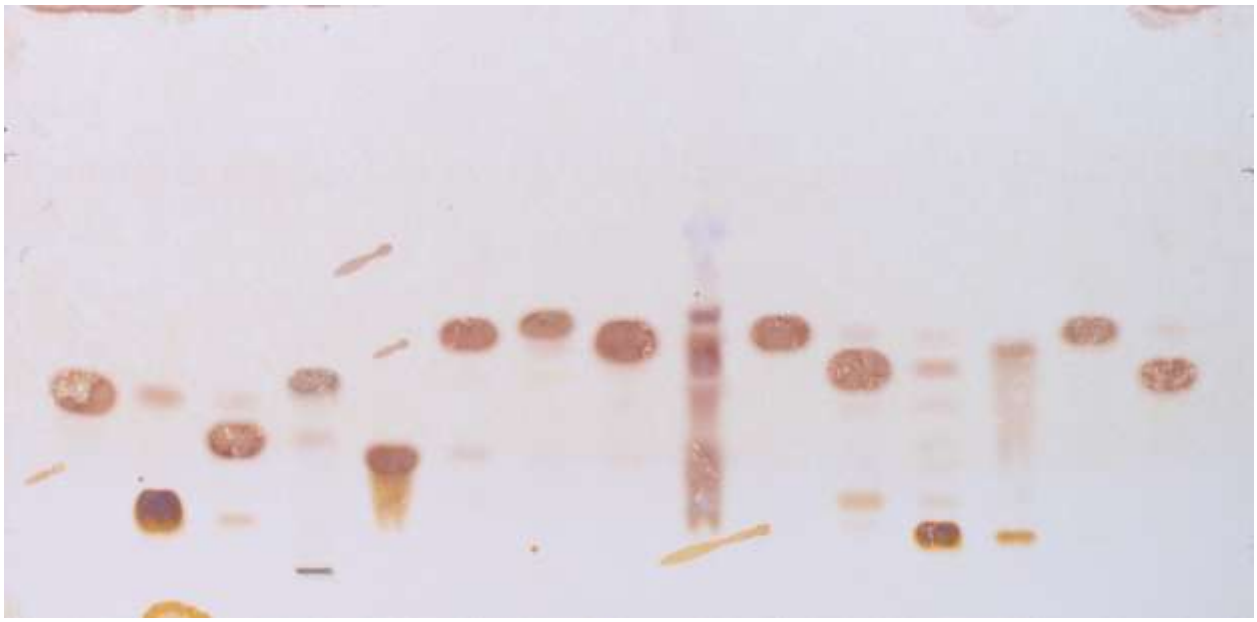


Fig. 4: HPTLC Plate of Std. Amino Acids and Seed's Ethanolic Extract under Visible Light

After derivatization the HPTLC plate showed more than 10 spots of Amino Acids. As described in Table 3 out of this certain amino acids identifies as, ornithine, arginine, alanine, glutamic acid, tyrosine, n-butyric acid, methionine, leucine and iso-leucine.

Their Rf value match with standard sample's Rf value.

Table 3: HPTLC Fingerprint Profile of *Amaranthus paniculatus* Seeds

Amino acids	Rf Value	
	A. paniculatus	Standard
Ornithine	0.12	0.12
Arginine	0.25	0.20
Alanine	0.32	0.38
Glutamic acid	0.32	0.30
Tyrosine	0.45	0.45
n-butyric acid	0.45	0.48
Methionine	0.58	0.55
Leucine	0.72	0.73
Isoleucine	0.72	0.72
-	0.78	-
-	0.83	-
-	0.97	-

Conclusion

These data and parameters have been investigated for *Amaranthus paniculatus* Seeds to lay down standards which could be useful to find the authenticity of this traditional medicinal system plant. These investigations may be useful to supplement existing information with regard to distinguish from substitutes and adulterants. In other words, the pharmacognostical features examined in the present study may serve as tool for validation of the raw material and for standardization of its formulations at herbal industrial level in the forth-coming days.

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