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Research Article

PHARMACOGNOSTIC AND PRELIMINARY PHYTOCHEMICAL INVESTIGATIONS OF *AMARANTHUS SPINOSUS* (LINN.) LEAVES

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ABSTRACT

Leaves of Plant *Amaranthus spinosus* Linn.were reported to possess good medicinal value in traditional system of medicine, the present investigation deals with Macroscopic, Microscopic and preliminary phytochemical investigation of leaves of *Amaranthus spinosus* which includes leaf constants, physiochemical parameters like ash values, extractive values and moisture content. The total ash, acid insoluble ash, water-soluble ash values and sulfated ash were observed to be 6.33%, 3.60%, 2.44% and 0.80% w/w respectively. Alcohol soluble and water-soluble extractive values of the leaves were observed to be 6.40%, 3.30%, respectively. powdered leaves were also subjected to fluorescence analysis with different chemicals. Phytochemical investigation of methanolic and petroleum ether extracts revealed the presence of Flavonoids, phytosterols, glycosides, tannins, phenolic compounds and carbohydrates. The main aim of the present investigation is to study the macro, microscopic and some other pharmacognostic characters and physicochemical standards of leaves of *Amaranthus spinosus* Linn. which could be used to prepare a monograph for the proper identification of the plant.

Keywords: *Amaranthus spinosus*, Phytochemical, Fluorescence analysis, Petroleum ether extract.

INTRODUCTION

Amaranthus spinosus Linn. (*Amaranthaceae*) [1]commonly called as Pig weed, is an annual herb found in throughout India and also many tropical countries .*Amaranthus* derived from the Greek word "amarantos" which means "unfading", a reference to the persisting color of certain *Amaranth* flowers.ethnomedicinally the plant is used as a source to treat several disorders such as the leaves are used as a laxative and applied as an emollient poultice to abscesses, boils and burns[2] .The juice of the root is used to treat fevers, urinary troubles, diarrhoea [3] , and dysentery[4]. The seed is used as a poultice for broken ribs [5]. Phytochemical Analysis revealed that *Amaranthus spinosus* contains a new coumaroyl flavone glycoside called spinoside [6], xylofuranosyl uracil, hydroxycinnamates, quercetin and kaempferol glycoside, betalains, betaxanthin, betacyanin ,phenolic compounds [7], amaranthine and isoamaranthine [8] , beta-sitosterol glycoside, campesterol [9], chemical analysis of leaves and stem gave hentriacontane and a-spinasterol , linoleic acid, rutin and beta-carotene[10], as prime phytoconstituents.

MATERIALS AND METHODS

Plant material collection and authentication

The leaves of plant *Amaranthus spinosus* Linn. were collected from the campus of Central Arid Zone Research Institute (CAZRI) Jodhpur, Rajasthan,India, in the month of June 2009 and were positively identified and confirmed by the botanist, Mr.A.sharma, Department of Botany, University of Rajasthan, Jaipur.a voucher specimen has been deposited (RUBAL 120616) in the herbarium of the botany department of the University of Rajasthan. The fresh mature leaves were used for the study of macroscopic and microscopic characters, whereas the dried uniform leaf powder was used for the extraction of active constituents of the plant, determination of ash value, extractive Values, loss on drying and phytochemical investigation.

Drying and pulverization

Leaves of *Amaranthus spinosus* were collected and cut into small pieces .it was shed dried and pulverized to mash size 22 and stored in air tight container for further use.

Extraction of powdered leaves

The powdered leaves were subjected to cold maceration process with petroleum ether (60-80) and methanol respectively. Both extracts were filtered individually, evaporated to dryness and stored in freezed condition for further use.

Pharmacognostic studies

Macroscopic studies

Morphological studies were done using simple microscope. The shape, apex, base, margin, taste and odour of leaves were determined.

Microscopic

Microscopic studies were done by preparing a thin hand section of midrib and lamina region of *Amaranthus spinosus* leaf. The section was cleared with chloral hydrate solution, stained with phloroglucinol and hydrochloric acid, and mounted with glycerin. A separate section was prepared and stained with iodine solution for the identification of starch grains. Powder of the dried leaves was used for the observation of powder microscopical characters. The powdered drug was separately treated with phloroglucinol and HCl solution. Glycerin and iodine solution were used to determine the presence of lignified cells, calcium oxalate crystals, trichomes and starch grains. As a part of quantitative microscopy, stomatal number, stomatal index, vein islet number and vein termination number were determined by using fresh leaves of the plant [11] .

Physiochemical investigation

The moisture content, total ash, water-soluble ash, acid-insoluble ash, sulphated ash, alcohol and water-soluble extractive values were determined as a part of its physiochemical parameters.the Powdered leaf parts were subjected to analysis under day/visible light and ultra violet light after treatment with various chemical as a part of Fluorescence analysis [12].

Phytochemical investigation

Petroleum ether and methanolic extracts were subjected to phytochemical analysis for the presence of various secondary phytoconstituents using standard chemical test.

RESULT AND DISCUSSION

The leaves of *Amaranthus spinosus* Linn were observed to be ovate or lanceolate shape, acute apex with entire margin, symmetrical base. (fig. 1) .The leaves of *Amaranthus spinosus* Linn. were found to have characteristic odour, bitter in taste (Table 1).

Fig. 1: Morphological features

Table 1: Morphological properties of leaves of *Amaranthus spinosus*

Properties	Observation
Colour	Green colour
Odour	Characterstic odour
Taste	Bitter in taste

In the microscopic studies, the leaf was found to be dorsiventral, and shows all the typical characteristics of leaf, as lamina part shows presence of epidermis, upper palisade and middle spongy parenchyma while midrib region shows upper and lower epidermis, collenchyma and centrally vascular bundle as phloem surrounds with the xylem. The leaves showed the presence of multicellular, slightly lignified covering trichomes on both the surfaces. (fig. 3).

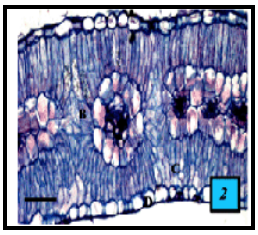

Fig.2: Epidermal cells

Microscopic study of powder revealed the presence of anomocytic stomata (fig. 4), oval, rounded starch grains, pitted xylem vessels, which are present in bundles and also clusters type of calcium oxalate crystals. Fig.2 shows the presence of epidermal cells. The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs.

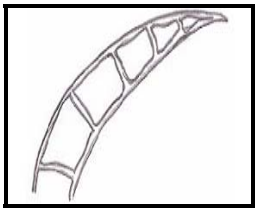

Fig .3: Multicellular covering trichome

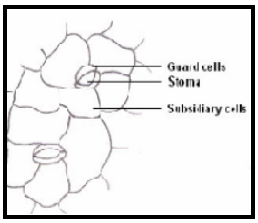

Fig. 4: Anomocytic stomata

Phytochemical tests for the presence of secondary phytoconstituents shows the following results (Table 5). Transverse section of fresh leaves of *Amaranthus* shows the presence of following characterstics (fig. 5).

The stomatal number, stomatal index, vein-islet number, vein-termination numbers are relatively constant for plants and can be used to differentiate closely related species. The results are depicted in (Table 2).

Table 2: Physiological parameters of leaves of *Amaranthus spinosus*

S. no	Parameter	Range	Mean
1.	Stomatal number		
	Upper epidermis	9-15	12
	Lower epidermis	-	
2.	Stomatal index		
	Upper epidermis	15-19	17
	Lower epidermis	-	
3.	Veinislet number	19-23	21
4.	Vein termination number	38-42	40
5.	Starch grains		
	Length	10.02-7.25-5.01	7.25
	width	6.68-6.38-5.01	6.38
6.	Calcium oxalate crystals		
	Length	7.51-6.26-5.34	6.26
	width	7.68-6.14-5.01	6.14

The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. The ash values, extractive values and moisture content of leaves were determined .The results are depicted in (Table 3).

Table 3: It shows the Physiochemical parameters of leaves of *Amaranthus spinosus*

S. no.	Parameter	Values (%)w/w
1.	Loss on Drying	2.30%
2.	Ash Values	
	Total Ash	6.33%
	Acid insoluble ash	3.60%
	Water soluble ash	2.44%
	Sulphated ash	0.80%
3.	Extractive Values	
	Water soluble extractive	6.40%
	Alcohol soluble extractive	3.30%
	Petroleum ether soluble Extractive	2.31%

results for fluorescence analysis of powdered drug with different reagents shows different colours. (Table. 4).

Table 4: It shows the Fluorescence analysis of powder of leaves of *Amaranthus spinosus*

Test	Fluorescence observed(color)
Powder +1N NAOH in methanol	No change in colour
Powder + 1N NAOH in water	Dark brown
Powder + 50% HCL	No change
Powder + 50% HNO3	Yellow
Powder + 50% H2SO4	Dark brown
Powder + Petroleum ether	No change
Powder + Chloroform	Brown
Powder + Picric acid	No change
Powder + 5% ferric chloride solution	No change
Powder + 5% iodine solution	No change

Table 5: It shows the preliminary Phytochemical screening of powder of leaves of *Amaranthus spinosus*

S.no	Test	Drug powder	Pet. ether extract	Methanolic extract
1.	**For Alkaloids**			
1 .1	Mayer's test	-ve	-ve	-ve
1.2	Dragendroff's test	-ve	-ve	Slightly +ve
1.3	Wagner's test	-ve	-ve	-ve
1.4	Hager's test	-ve	+ ve	+ve
2. For carbohydrates				
2.1	Molisch test	+ve	+ve	+ve
2.2	Fehling's test	+ve	_ve	-ve
2.3	Benedict's test	+ve	+ve	+ve
2.4	Iodine test	-ve	-ve	-ve
3. For proteins				
3.1	Biuret test	-ve	-ve	-ve
3.2	Xanthoprotein test	-ve	-ve	-ve
4. For flavonoids				
4.1	Shinoda test	-ve	-ve	-ve
4.2	Lead acetae test	+ve	+ve	+ve
4.3	Reaction with alkali/acid	-ve	+ve	+ve
5. For heavy metals				
5.1	With copper sulphate penta hydrate	-ve	-ve	-ve
5.2	With lead acetate trihydrate	-ve	+ve	+ve
6. For tannins and phenolic compounds				
6.1	With lead acetate	+ve	+ve	+ve
6.2	With pot.dicromate sol.	+ve	-ve	+ve
6.3	With fecl3	-ve	-ve	+ve
6.4	With potassium permagnate	-ve	-ve	-ve
7.	**For gum and mucilage**			
7.1	With ruthentium red	-ve	+ve	-ve
7.2	Swelling property	-ve	-ve	-ve
8.	**For fixed oils and fats**			
8.1	Spot test	-ve	-ve	-ve

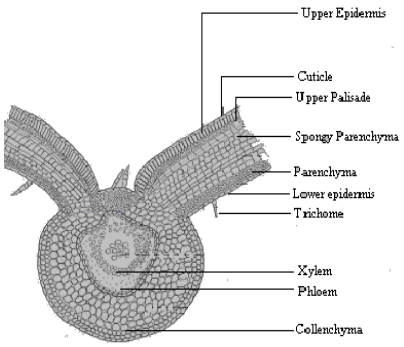

Fig. 5: T.S of *Amaranthus spinosus* fresh leaves

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