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PHARMACOGNOSTIC STUDIES ON STEM AND LEAVES OF AMARANTHUS SPINOSUS LINN.

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ABSTRACT: The seed is used as a poultice for broken bones. It is used internally in the treatment of internal bleeding, diarrhoea and excessive menstruation. The root is known as an effective diuretic. In South-East Asia a decoction of the root is used to treat gonorrhoea and is also applied as an emmenagogue and antipyretic. The Nepalese and some tribes in India apply A. spinosus to induce abortion. In Thai traditional medicine, A. spinosus is used to treat diarrhea. The root is also used for toothaches. In many countries, including those in Africa, the bruised leaves are considered a good emollient and applied externally in cases of ulcerated mouths, eczema, burns, wounds, boils, earache and hemorrhoids

The leaves are also used for gastroenteritis, gall bladder inflammation, absesses, colic menorrhagia, arthritis and for the treatment of snakebites. The plant ash in a solution is used to wash sores. The plant sap is used as an eye wash to treat ophthalmia and convulsions in children. In Malaysia, A. spinosus is used as an expectorant and to relieve breathing in acute bronchitis. In mainland South-East Asia, it is also used as a sudorific, febrifuge, an antidote to snake poison, and as a galactagogue. During the rainy season which is also malaria endemic season, A. spinosus bark decoction is taken in a volume of about one liter three times a day to ward off malaria.

Key words: *Amaranthus spinosus linn*, Pharmacognostic study, Stem and Leaves

INTRODUCTION

Amaranthus spinosus grows annually as an erect, monoecious herb, up to 100–130 cm tall, much branched; stem terete or obtusely angular, glabrous or slightly pubescent, green or variably suffused with purple . The leaves alternate and are simple without stipules; petiole is approximately as long as the leaf-blade; The blade shape is ovate-lanceolate to rhomboid, 3.5–11 cm × 1–4.5 cm, acute and often slightly decurrent at base, obtuse, rounded or slightly retuse and often short mucronate at apex, entire, glabrous or slightly pubescent on veins when young. The inflorescence consists of dense clusters, lower ones are axillary, higher ones often collected in an axillary and terminal spike which is often branched in its lower part; axillary clusters are usually armed with very sharp spines up to 2 cm long. Its flowers are unisexual, solitary in the axil of a bract, subtended by 2 bracteoles; bracts and bracteoles scarious, mucronate from a broad base, shorter or as long as the perianth; male flowers are usually arranged in a terminal spike above the base of the inflorescence, green; tepals 5 or in male flowers often 3, free, subequal, ovate-oblong to oblong-spatulate, up to 2.5 mm long, very convex, membranous, with transparent margins and green or purple median band; male flowers with 5 stamens about as long as tepals; female flowers with superior, oblong ovary, 1-celled, styles 2–3, ultimately recurved.

The fruit is ovoid shaped with a short inflated neck below the style base, circumscissile a little below the middle or indehiscent. The seed is about 1 mm in diameter, shiny, compressed, black or brownish-black in colour.

The Chinese use A. spinosus as a traditional medicine to treat diabetes. The seed is used as a poultice for broken bones. It is used internally in the treatment of internal bleeding, diarrhoea and excessive menstruation. The root is known as an effective diuretic. In South-East Asia a decoction of the root is used to treat gonorrhoea and is also applied as an emmenagogue and antipyretic. The Nepalese and some tribes in India apply A. spinosus to induce abortion[1,2]

Amaranthus spinosus contains 7-p-coumaroyl apigenin 4-O-beta-D-glucopyranoside, a new coumaroyl flavone glycoside called spinoside, xylofuranosyl uracil, beta-D-ribofuranosyl adenine, beta-sitosterol glucoside, hydroxycinnamates, quercetin and kaempferol glycosides, betalains; betaxanthin, betacyanin; amaranthine and isoamaranthine, gomphrenin, betanin, b-sitosterol, stigmasterol, linoleic acid, 0.15% rutin and beta-carotene[3,4,5]. The carbohydrate content is 1.16 g/100 g leaves, energy 27 kcal, moisture 91 g, protein 4 g, fat 0.6 g, fiber 2.48 g, ash 2.76 g.[6]

Iron (38.4 mg/100 g dry weight), calcium (968.7 mg/100 g dry weight), magnesium (912.4 mg/100 g dry weight), phosphorus (816.3 mg/100 g dry weight), manganese (6.8 mg/100 g dry weight), copper (1.2 mg/100 g dry weight), zinc (6.8 mg/100 g dry weight)[7].

In Thai traditional medicine, A. spinosus is used to treat diarrhea[8].The root is also used for toothaches[9]. In many countries, including those in Africa, the bruised leaves are considered a good emollient and applied externally in cases of ulcerated mouths, eczema, burns, wounds, boils, earache and hemorrhoids

The leaves are also used for gastroenteritis, gall bladder inflammation, absesses, colic menorrhagia, arthritis and for the treatment of snakebites[10]. The plant ash in a solution is used to wash sores. The plant sap is used as an eye wash to treat ophthalmia and convulsions in children. In Malaysia, A. spinosus is used as an expectorant and to relieve breathing in acute bronchitis. In mainland South-East Asia, it is also used as a sudorific, febrifuge, an antidote to snake poison, and as a galactagogue[11]. During the rainy season which is also malaria endemic season, A. spinosus bark decoction is taken in a volume of about one liter three times a day to ward off malaria[12]**.**

MATERIALS AND METHODS

The whole plants of *Amaranthus spinosus* Linn were procured from different places of Purulia, District, WB and authenticated by the Botanical survey of India, Shibpur, Howrah, WB and also by Botany Department, B.B.College,Asansol,WB, India. A voucher specimen (NO-CHN/I-I/2008/Tech. II/) was retained in our laboratory for further references.The plant material was dried in sunlight,pulverized,passed through sieve no.40 and stored in air tight container and used for further extraction.

METHODS

Macroscopic and microscopic analysis

The macroscopic characters such as colour ,odour, taste, nature, texture were studied for morphological investigation.

Isolation of epidermal layers

35 leaves were immersed in chloral hydrate and methanol for 2 days to remove debris and chlorophyll.The solution was heated gently so that the debris and chlorophyll are removed quickly.After that 1 sqmm from 35 leaves were cut and immersed in 20% chromium trioxide for maceration.[13]

Microscopic study

Peeling of the macerated leaf was done with the help of finger and blade.1 sqmm of the peeling was cut and stained in 1% aqueous solution of safranin for 3 min.Excess stain was rinsed off with water.The stained specimen was then mounted in glycerine for observation under Olympus microscope.

For anatomical studies customary technique of microtmy was followed[14]. Paraffin sections of 10µm thick were stained with safranin – fast green. Photomicrography were taken with Gupta college lab photo – microscopic unit .The quantitative microscopy was studies as per the procedure given by Wallis[15]. and P.K.Lala[16]. The powder analysis has been carried out according to the method of Brain and Turner[17].

Physico – chemical studies

The ash values, extractive values and loss on drying were performed according to the officinal methods prescribed in Indian pharmacopeia[18] and the WHO[19] guidelines on quality control methods for medicinal plants materials. Fluorescence analysis was carried out according to the method of Chase and Pratt[20] and Kokoski [21] .

Preliminary phytochemical screening

The dried leaves were extracted with petroleum ether, chloroform, ethylacetate and methanol. The behavior of powder with various chemical reagent and preliminary chemical tests for various extracts were also carried out according to the standard procedures described by Khandelwal[22] and Harborne[23].

RESULTS

Macroscopy

Amaranthus spinosus grows annually as an erect, monoecious herb, up to 100–130 cm tall, much branched; stem terete or obtusely angular, glabrous or slightly pubescent, green or variably suffused with purple. The leaves alternate and are simple without stipules; petiole is approximately as long as the leaf-blade; The blade shape is ovate-lanceolate to rhomboid, 3.5–11 cm × 1–4.5 cm, acute and often slightly decurrent at base, obtuse, rounded or slightly retuse and often short mucronate at apex, entire, glabrous or slightly pubescent on veins when young. The inflorescence consists of dense clusters, lower ones are axillary, higher ones often collected in an axillary and terminal spike which is often branched in its lower part; axillary clusters are usually armed with very sharp spines up to 2 cm long. Its flowers are unisexual, solitary in the axil of a bract, subtended by 2 bracteoles; bracts and bracteoles scarious, mucronate from a broad base, shorter or as long as the perianth; male flowers are usually arranged in a terminal spike above the base of the inflorescence, green; tepals 5 or in male flowers often 3, free, subequal, ovate-oblong to oblong-spatulate, up to 2.5 mm long, very convex, membranous, with transparent margins and green or purple median band; male flowers with 5 stamens about as long as tepals; female flowers with superior, oblong ovary, 1-celled, styles 2–3, ultimately recurved. The fruit is ovoid shaped with a short inflated neck below the style base, circumsessile a little below the middle or indehiscent. The seed is about 1 mm in diameter, shiny, compressed, black or brownish-black in colour.

Microscopic features of the leaves

Microscopical studies are useful to establish the botanical identity for the valuable herbal drugs, which forms the basis for the identification and determination of adulterants.Stomatal frequency was done by using field of view at *10 objective as a quadrant, frequency of stomata type was expressed as percentage occurance of each type in the total of 35 field of view at *10 objective on microscope. Terminology used in stomatal types followed those of Dilcher [23] and Metcalf and Chalk [24].The leaf is dorsiventral, hypostomatic and mesomorphic. It has thick midrib projecting both adaxially and abaxially (fig 1). The epidermal layer of thin midrib consists of small, squarish, thick walled cells with prominent cuticle, the cells are with prominent cuticle and the cells are 22 µm thick.

Fig.1 *Amaranthus spinosus* Linn.

The palisade layer of the lamina extend up to the lateral part of the hump (Fig.1) The lower semicircular midrib has parenchymatous ground tissue. The cells are wide thin walled, angular and compact. The vascular system of the midrib consists of an adaxially flattened closed cylinder of xylem and phloem ; with in the cylinder are two small rectangular segments of vascular bundles. The outer cylinder has a thin layer of xylem fibres and short radial files of narrow, thick walled angular xylem elements and outer continuous zone of phloem.Medullary accessory bundles are collateral with xylem elements facing the adaxial side and phloem elements placed toward the centre. The vascular cyclinder is 170 µm thick. The xylem elements are 20µm wide. The lamina has wide radially oblong thick walled adaxial epidermis with prominent cuticle. The epidermal cells of abaxial surface and adaxial surface is isodiametric and anticlinal wall of cell wall is wavy on both surface.The adaxial epidermis is 40 µm thick. The abaxial epidermis has comparatively small cells which are squarish in shape, the cuticle is thicker; stomata are present on the lower epidermis. Stomata mostly of anomocytic, anisocytic type are of less frequent. Trichomes are uniseriate.Trichomes dendity is 1.75 ± 2.08(mm^2) on abaxial surface and 2.82± 2.35(mm^2) on adaxial surface.There are two layers of palisade cells along the upper part. The cells are wide, cyclindrical and the palisade zone in 60µm in height. The spongy parenchyma cells are in 4 (or) 5 rows. The vascular strands of the lateral veins are circular with thick cylinder of fibers and small central case of xylem and phloem.

Quantitative microscopy

Quantitative microscopic data are found to be constant for a species. These values are especially useful for identifying the different species of genus and also helpful in the determination of the authenticity of the plant. The study of the leaf constants showed that the average stomatal number is 116mm^2, the stomatal index is 2.30mm^2. The vein islets number and vein termination numbers are 48.5mm and 98.3mm/sqmm respectively. The palisade ratio is 9.3. The microscopic linear measurement of the trichomes showed that the length of the trichome is 44-65µm and the width is around 20 -24µm (Table no1).

Table-1 Data showing the values of quantitative microscopy

S.No.	Method	Range	Average
1	Stomatal no	115-117mm^2	116mm^2
2	Stomatal index	23-25 mm^2	23.0 mm^2
3	Vein islet no	47-49 mm^2	48.5 mm^2
4	Vein termination	97-99 mm^2	98.3 mm^2
5	Palisade ratio	9-11	9.3
6	Linear measurement Length Width	 44-65µm 20-24 µm	 51.2 µm 22 µm

Powder characters:

The powder characters of a drug are mainly used in the identification of the drug in the powder from. The leaf powder was light green in colour with strong and characteristic in taste on microscopical examination the powder showed anisocytic stomata, unicellular covering trichomes.

Physico-chemical constants

The physico-chemical parameters are mainly used in judging the purity and quality of the drug. Ash values of a drug give an idea of the earthy matter or inorganic composition or other impurities present along with the drug. The ash values of the powdered leaves evealed a high percentage of sulphated ash. Extractive values give an idea about the chemical constituents present in the drug as well as useful in the determination of exhausted or adulterated drugs. The results suggest that the powdered leaves have high water soluble extractive value. The loss on drying reveals the percentage of moisture present in the drug are also studied and presented in (Table no 2).

Table-2 Data showing physico-chemical standard values of leaves of *Amaranthus spinosus* Linn

S.No.	Total ash%	Water soluble ash%	Acid insoluble ash%	Water soluble extractive	Alcohol soluble extractive	Loss on drying
1	15	7.5	2.25	1.908	1.646	0.3
2	14.2	7.1	2.12	1.732	1.526	0.2
3	16.3	7.8	3.20	1.842	1.653	0.5
4	13.5	6.7	2.10	1.632	1.434	0.6
5	15.6	7.6	2.27	1.942	1.746	0.2
Min	13.5	6.7	2.10	1.632	1.434	0.2
Ave	14.9	7.34	2.38	1.811	1.601	0.3
Max	16.3	7.8	3.2	1.942	1.746	0.6

Fluorescence analysis of drug powder and extracts:

The fluorescence analysis of powdered leaves was studied in both UV and day light. (Table No. 3 & 4).

Table: 3 Fluorescence analysis[20-21] of powder of *Amaranthus spinosus* (whole plant) with various chemical reagents.

Reagents	Visible Light	UV Light (254nm)
Powder as such	No change	No change
Powder + 1N NaOH	Pale Yellow	Colour less
Powder + 1N NaOH in ethanol	Pale Yellow	Colour less
Powder + Ethanol	Pale Yellow	Colour less
Powder + HNO_3 + NH_3 Solution	Brown	Colour less
Powder + 50% HNO_3	Light Brown	Pale Brown
Powder + HCL	Light Green	Brownish Green
Powder + H_2SO_4	Deep Brown	Light Brown
Powder + Picric acid Solution	Pale Yellow	Bright yellow
Powder + Glacial acetic acid	Light Yellow	Colour less
Powder + Concentrated HNO_3	Brick Red	Light Brown

Table: 4 Analysis of different solvent extracts of *Amaranthus spinosus* (whole plant) under UV & Visible Light

Extract	Observation	
	Visible Light	UV Light (254nm)
Petroleum ether (60-80°C)	Yellow	Orange Red
Chloroform	Dark Brown	Deep Reddish Orange
Ethyl acetate	Greenish Brown	Reddish Orange
Ethanol	Dark Green	Orange
Water	Yellowish Brown	Light Green

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