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RESEARCH ARTICLE

NOVAL TRAITS FOR IDENTIFYING AMARANTHUS SPINOSUS L. USING SYSTEMATIC AND BIOCHEMICAL ANALYSIS.

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Abstract

Amaranthus spinosus is commonly known as spiny amaranth and is distributed all around the Asian continent as an annual weed. Though it is seen as a weed the nutritive quality of the species is high. This paper attempts to explain the various biochemical composition of the plant qualitatively and quantitatively. Qualitative analysis carried out suggests the better solvent which could be used for its effective extraction. This paper also puts lights into the taxonomical traits and anatomical characters of the species.

Introduction:-

The genus Amaranthus, a widely cultivated taxonomic group, consists about 60–70 species [1]. Collectively known as Amaranth, it is a cosmopolitan genus of herbs belonging to the family Amaranthaceae[2]. Most of amaranth species are summer annual weeds and are commonly referred to as pig weed [3]. A rich array of poems and folklore has hints about the flower of amaranth which does not fade easily. Its etymology as well, has traces to its everlasting flower, with the word Amaranth, coming from the Greek word Amaranthon, which means 'one that does not wither [4]. Throughout history, Amaranth (Amaranthus sp.) has been highly priced as a consumable. The Incas, Mayans and Aztecs used it as a staple food[5]. Amaranthus spinosus L. is an erect, monoecious, perennial, up to 1 m, stems is terete or obtusely angular, glabrous or slightly pubescent, green, reddish-brown, glabrous, and branched. The leaves of A.spinosus are alternate and simple without stipules; petiole is approximately as long as the leaf blade. The blade shape is ovate-lanceolate to rhomboid, acute and often slightly decurrent at base, obtuse, rounded and often short mucronate at apex, entire, glabrous or slightly pubescent on veins when young [6]. The inflorencence of the species possess terminal and axillary spike, which are, erect, slender and elongated, with remote axillary spikes at base, present in lower clusters [7]. The anatomy of A.spinosus shows the presence of vascular bundles in the pith, known as medullary bundles, in addition to the normal ring of vascular bundles [8]. The presence of medullary bundles has been reported as an 'anomaly', which according to Esau is 'a growth pattern which occur less common [9]. The nutritional composition of spiny Amaranthus is carotene (11.94), Vitamin C (43 mg), calcium (374 mg), carbohydrates (5.0g), protein (5.3g), fat (0.1g) 100g of the edible portion [10].

Materials and Methods:-

Taxonomical studies:-

The fresh plant within one week of flowering was collected. It was identified and was subjected to taxonomical identification of different characters. The plants were worked out with the help of The Flora of The Presidency of Madras (Gamble) Volume 2.The characters and variations were noted. The herbariums of the plant species were also prepared.

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Anatomical studies:-
Some mature and fresh parts of the leaves, stems and roots of the species among werecollected. With the help of a razor blade different transverse sections of stem and root were obtained by cutting along the radial plane of a cylindrical portion of the stem and root. Leaf was dissected with the help of papaya pith. All the fine dissections were kept in watch glass along with water. The sections were mounted as performed by Omosun, with modifications. The obtained sections were mounted temporarily; photographed using Leica photomicroscope and the characters were noted. Safranin was used to stain the sections and glycerine was used as the mountant. The sections were then photographed.

Biochemical analysis:-
The fresh plant material was extracted in water, acetone, methanol, and chloroform and the presence of various biochemical entities were checked qualitatively. The qualitative analysis was done by the procedures of Sadashivam and Manickam[11]. Qualitative analysis for carbohydrates was performed by Molisch's test, iodine was noted by iodine test. The presence of reducing sugars was confirmed by Benedict's test. Biuret test and Ninhydrine test were performed for the confirmation of proteins and amino acids respectively. Ferric chloride test were performed for the confirmation of tannin. Qualitative analysis of flavonoid and phenolics were also carried out.

Quantitative analysis for biochemical constituents in plant samples:-
The total moisture content was determined based onJim et al. in1999. The differences in weights of fresh samples and oven dried samples were determined to find out the total moisture content. Carbohydrate content was measured spectrophotometrically (Hedge& Hofreiter, 1962) [10] using anthrone method. Glucose was used as the standard. Reducing sugars were determined based on Miller, 1972.Starch contentwasestimated spectrophotometrically using anthrone reagent (Hedge &Hofreiter,1962)[10]. Determination of crude fiber was carried out by the method of Maynard, (Ed) (1970). Protein determination was carried out based on Lowry et al., 1951[12]. Determination of amino acid was carried out following the protocol of Moore &Stein, 1948.Total phenol content was detected spectrophotometrically based on Malick& Singh, 1980[7]**.** The tannin content was determined by Vanillin Hydrochloride method. Flavonoid content was determined spectrophotometrically.

Results and Discussions:-

The taxonomical characters of A.spinosus suggests that it an erect annual herb with green spiny stem which is sparsely pubescent. It was earlier reported that the leaf is glaborous. In the present study presence of small microscopic hairs in the leaf suggests it to be sparsely pubescent and hence a new trait in identifying the species. The inflorescence is catkin type and is green in colour is similar to the one reported by Xian S,2003 but the presence of spine throughout the length of the spike is also reported in the present study. The 5 lobed structure of the periyanth can also be used as a major lead in identifying the species.

Table 1:- Description of vegetative characters in A.spinosus.

Vegetative characters	DESCRIPTION
Habit	Erect annual herb
Stem	Green, erect, branched, glaborous, spiny, terate
Leaf	Alternate, sparsely pubescent, ovate margin,
Root	Tap root, highly branched

Table 2:- Description of reproductive characters in A.spinosus

Reproductive characters	DESCRIPTION
Inflorescence	Terminal, axillary & nodal, green spikes, catkin type, spines present
Flower	Male flower in terminal spike, female flowers only in nodes, periyanth 5 lobed, bract length equals periyanth length

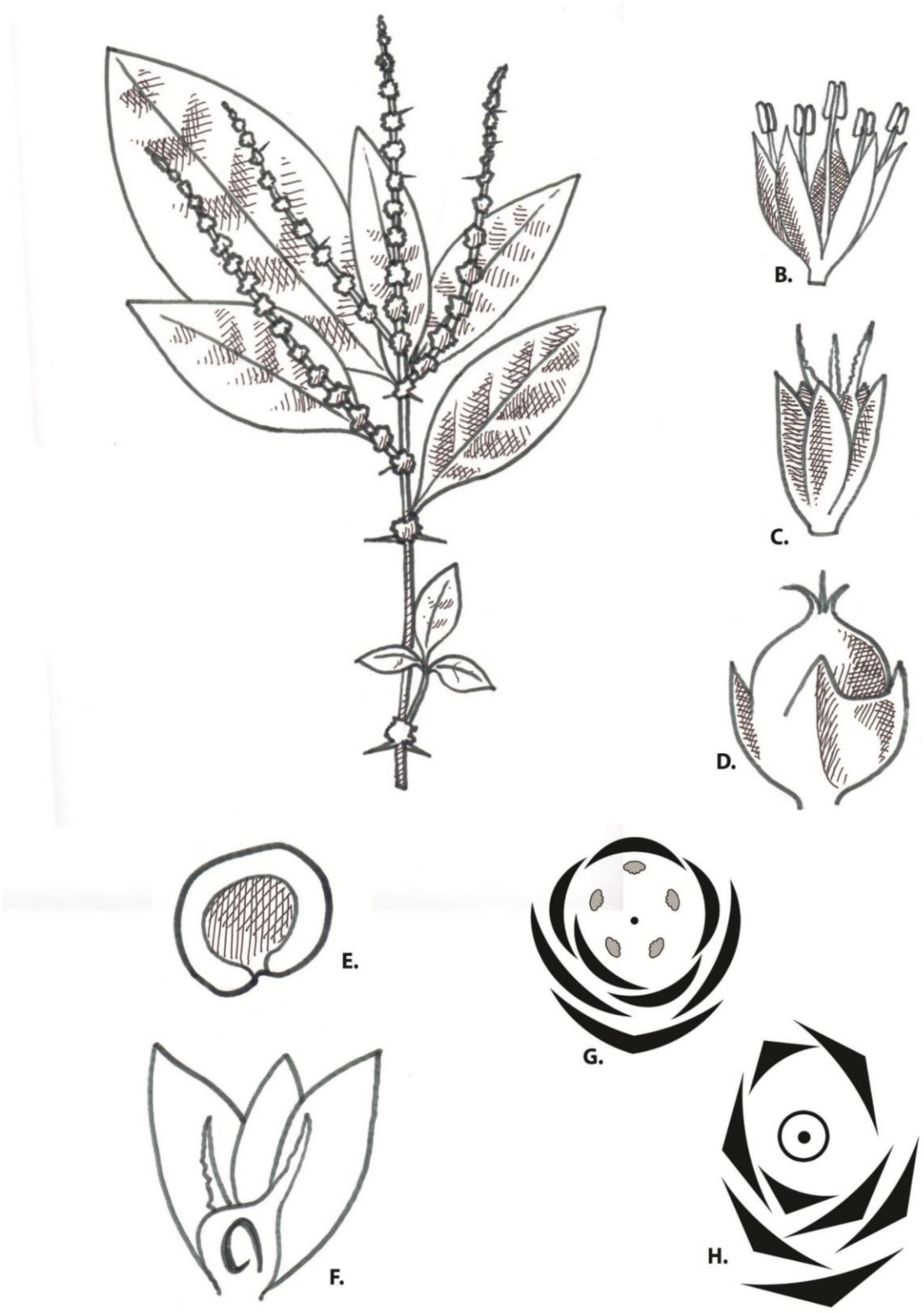


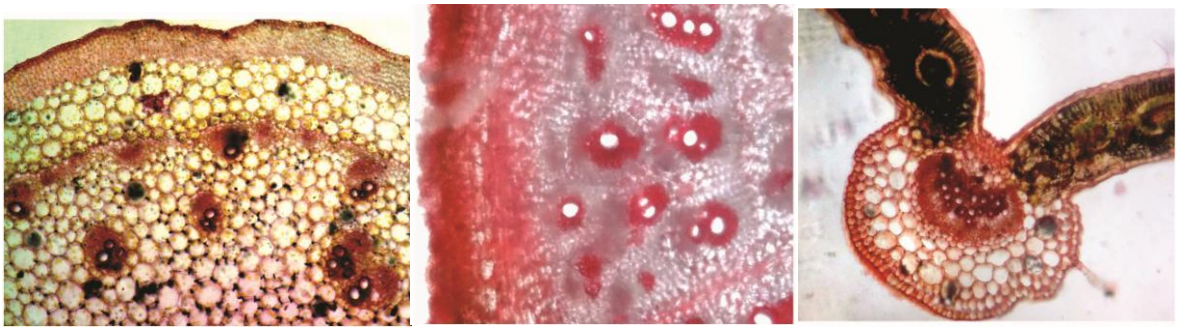
Figure 1: *A spinosus* L.

A. Habit **B.** Male flower **C.** Female flower **D.** Fruit **E.** Seed V.S **F.** Female Flower L.S **G.** Floral diagram: Female flower **H.** Floral diagram: Male flower

The stem anatomy shows the presence of a single layered epidermis with papilose elongated cells. The cortex is composed of lacunar collenchyma. The vascular bundles are scattered and numerous. The pith region has the presence of sandy crystals. The leaf anatomy suggests that it has the presence of multicellular epidermal hairs. The vascular bundle in the midrib region is C shaped with a large apical vascular bundle.The results were almost similar to the one reported by Chandurkar 1983 except for the presence of lacunar collenchyma in the cortex region.

Table 3:- Anatomical description of A.spinosus

Vegetative parts	Description
Stem	Epidermis:1-2 layered papilose elongated cells Cortex:8-10 layered lacunar collenchyma Vascular bundles scattered Pith zone contains sandy crystal
Leaf	Epidermal hairs present only abaxial surface Vascular bundle C-shaped
Petiole	5 C shaped vascular bundle with basal bundles small and apical one large
Root	Secondary xylem more than 7 and arranged in two groups. Distribution of parenchyma



(a) (b) (c)

Figure 2: *A. spinosus* L. S (a) Stem (b) Root (c) Leaf

Ali Khoddami 2013[13] reported that carbohydrate starch reducing sugar proteins and amino-acids can be extracted effectively by polar solvents. The result obtained here was almost similar with slight variations like A.spinosus showed a positive result with acetone which is a non- polar solvent butshowed absence of starch when extracted with polar solvents. According to Sowmya andLakshmideviin 2013[14] carbohydrate and proteins were extracted effectively only in ethanol, contradictory to this report, the present study shows that carbohydrates and proteins could be extracted using aqueous and acetone also.Tannin, flavonoids and phenolic were qualitatively estimated and the result shows that tannin was present only in alcoholic extraction of sample. All the other polar and non- polar solvents showed complete absence of tannin when extracted. Phenols and flavonoids were extracted using alcohol and acetone. Rest of the solvents shows its complete absence. This was similar to the one reported by Okwu, 2004. According to the qualitative analysis it could be concluded that polar compounds could be extracted with polar solvents and non-polar compounds with non-polar solvents.

Quantitative analysis shows that A. spinosus has protein and amino-acids in large concentration than the other biochemical compounds.Soluble sugar content as reported by Begum in 2000 is 5 mg/100mg of tissue. The present study gave almost similar value but by a marginal increase of 0.55 mg. Starch content was in close proximity with the one reported by Costea et al., 2001. The protein content and amino acid content was higher than the one reported by Begum in 2000[15]The result obtained was also in close proximity with the one reported by Haque in 1985.Secondary metabolites obtained were present in minimal amounts in both the species. Tannin, flavonoid and total phenolic content were much lower than the concentration reported by Akubugwo *et al*in 2007. According to his report the values for flavonoid, tannin and phenols were 0.83, 0.49 and 0.35mg/100g of sample respectively. The

present study shows that the content of flavonoid gave a lower value of 0.0002mg. Tannin content and total phenolic content was also lower than the one reported earlier and ranged from 0.0001 and 0.0021 respectively. The increase in tannin, flavonoid andtotal phenolic content in both the species treated with inorganic fertilizer can be attributed as a stress response of the plant against salt stress, caused by the usage of inorganic salts. With respect to the experimental data, it can be concluded that plants treated with organic nutrients make a more nutritious and filling diet

Table 4:- Quantitative analysis of different biochemical components (100mg of sample) in A.spinosus.

Biochemical compounds	Concentration /100mg of sample
CARBOHYDRATES	5.55
REDUCING SUGARS	3.19
STARCH	3.56
PROTEIN	7.14
AMINO ACID	6.16
TANNIN	0.0001
FLAVONOIDS	0.0002
PHENOLICS	0.0021

Table 5:- qualitative test with different solvents

TEST	Aqueous	Ethanol	Petroleum ether	Acetone
Molish's test	+	+	-	+
Iodine test	-	-	+	+
Benedict test	+	+	_	-
Biuret test	+	+	-	+
Ninhydrine test	+	+	-	+
Ferric chloride test	-	+	-	-
Test for phenolics	-	+	-	+
Test for flavonoids	-	+	-	+

Conclusions:-

The taxonomical character of A.spinosus suggests certain new traits for identifying the species. In the present study presence of small microscopic hairs in the leaf suggests it to be sparsely pubescent. The presence of spine throughout the spike and the 5 lobed structure of the periyanth can also be used as a major lead in identifying the species. The anatomy of the species were similar to the previous reports except for the fact that cortex region of the stem showed the presence of lacunar collenchyma. According to the qualitative analysis it could be concluded that polar compounds could be extracted with polar solvents and non-polar compounds with non-polar solvents.Quantitative analysis shows that A. spinosus has protein and amino acids in large concentration than the other biochemical compounds. Increased concentration of nutritive compounds and decreased concentration of secondary metabolites suggests it to be a more filling diet.

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