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Research Article

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Morphological and phytochemical studies of *Suaeda maritima* (L.) Dumort growing along the coastal belt of Purba Medinipur District, West Bengal, India in search of the prospective variation

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ABSTRACT

Suaeda maritima (L.) Dumort of the family Chenopodiaceae is an annual succulent mangrove herb. This annual salt marsh is quite regularly used by the local people for food and pharmaceutical. This species has been cursorily noted to have variation in some morphological characters. Earlier reports indicated the presence of triterpenoid e.g. alpha amyrin in some species of *Suaeda*. However, no report on the variation in the quantity of it in this species was presented. The present study has furnished an account of subtle variation in morphology of this herb growing on different sites in the area under study. It also shows a difference in the amount of alpha amyrin in the plant individuals of different places, revealed through the HPTLC study. Morphological variations have been noted mostly in respect of the characteristics of the stem and leaf of the species.

KEYWORDS: *Suaeda maritima*, Morphological diversity, Alpha amyrin, Chromatographic analysis

INTRODUCTION

Mangrove vegetations grow along with the coastal belts of tropical and sub-tropical regions,usually between 25° N and 25° S latitude throughout the world (Tomlinson,1986). Annual succulent herbs of the species of *Suaeda* grow naturally in soils having a high concentration of salt of mangroves (Untawale, 1984). *Suaeda maritima* grows luxuriantly along the coastal belt of Purba Medinipur district of West Bengal in India, right from Hijli-Sarif of Khejuri to Udaypur of Digha (Das, 2015). This range of coast in Purba Medinipur district is lying between 21° 51´27´´N to 21°36´5´´N latitude and 87°29´88´´E to 88°12´40´´E longitude. There is a record of its use as a vegetable and also in curing malady (Trease & Evans, 2002) and such uses are also noticed among the local people of the area under study. Though this herb grows almost continuously along the entire stretch of the region mentioned here, shows subtle variation in gross morphology along the site of its growing. Such diversity might be more due to the variation in the chemical and physical properties of the soil along the region, rather than the genetic property of the plants for this contiguously and naturally growing herb. Such variation might also have a bearing on the biochemical constitution of them, too. Early literatures recount several different phytochemical compounds like triterpenoid, sterols, alkaloids, acids, glycosides (Krishchenko *et al.*,1984; Kapadia *et al.*, 1985; Miftakhova *et al.*, 1999), proteins and amino acids (Marie, 1965) to occur in this species. Alpha amyrin, a pentacyclic triterpenoid, a biomolecule of worth, has earlier been reported to occur in this species (Ghosh *et al.*, 1985). α and β amyrins are two structural isomers possessing a wide spectrum of pharmaceutical and biological functions like, anti-microbial, insecticidal (Bandeira *et al.*, 2006; Ekalu *et al.*, 2019), anti-arthritic, anti-inflamatory, anti-nociceptive, anti-depressant, anti-hyperglycemic (Siani *et al.*,1999; Oliveira *et al.*,2004a; Oliveira *et al.*,2005b; Aragao *et al.*, 2006; Aragao *et al.*, 2007; Holanda *et al.*, 2008; Melo *et al.*, 2010; Barros *et al.*, 2011; Melo *et al.*,2011; Santos *et al.*, 2012; Aragao *et al.*, 2015; Carvalho *et al.*,2017; Pinto *et al.*, 2017), anti-ulcer, gastroprotective

(Oliveira *et al.*, 2004b; Oliveira *et al.*, 2005a; Prabhakar *et al.*, 2017). Any variation in the amount of this secondary metabolite occurring between different plant individuals of this species would provide scope for selection of better producer among them, aspect of economic significance. With this matter as an objective, the present study has scrutinized and revealed subtle morphological and biochemical diversities amongst the individuals of the species growing in the coastal area of West Bengal.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Aerial parts of *Suaeda maritime*, collected from the coast of Bay of Bengal in Purba Medinipur district of the state of West Bengal, India, were taken as study material. The plant samples collected from Bankiput has been designated as FSB and that from Sankarpur as FSS in this literature. Plant samples were collected at the end of monsoon from Sankarpur (21°61´97´´N, 87°57´22´´E) and Bankiput (21°76´67´´N, 87°86´65´´E) situated 41.5 km apart, along the coastal belt of Purba Medinipur district, at their flowering and fruiting stage. Herbarium was prepared with the collected sample according to Jain and Rao (1977) and Brummit and Powell (1992). The herbarium was identified and authenticated by the Central National Herbarium (CAL) of Botanical Survey of India (voucher specimen number CNH//2015/Tech.II/17/299).

Morphological Studies

Morphological studies were carried out with freshly collected whole plants. The studies included habits of the plant, leaf, stem, root, flower, fruit and seed. Along with gross morphological study with bare eyes, different plant parts like leaf, flower, fruit and seed were studied and measured under a microscope.

Biochemical Studies

For the purpose of biochemical study the aerial parts of the plant were chopped into pieces and dried in shade at room temperature and were pounded to powder in a mechanical grinder. Twenty grams of the dried aerial plant part from each sample was weighed and poured into 150 mL of methanol solvent. The mixture was stirred every 30 min for 3 h and thereafter kept for two days (Ali *et al.*, 2001; Mammen *et al.*, 2010; Reich & Schibli, 2011). The extracts of the plants from two sites of the collection were filtered separately using Whatman No 1 filter paper at 25°C temperature. The extracts obtained, thus, were concentrated to one third of their original volume by placing in a rotary evaporator. The concentrates were transferred into reagent bottles and stored in a refrigerator for HPTLC analysis.0.50 mL of this solution was diluted up to 10.0 mL by methanol to obtain the working standard solution of alpha-amyrin (Stahl, 1969). Standard alpha-amyrin (purity 99.3%), from Sigma-Aldrich Chemie GmbH (Aldrich Division, Steinbeim, Germany) was used as reference. A stock solution of this standard chemical 1000 µg/mL was prepared.

High Performance Liquid Chromatography Analysis

Chromatography was performed on 20 cm × 10 cm TLC aluminum precoated silica gel $60F_{254}$ plate, with 200 µm layer thickness (E. Merck, Mumbai, India). Standard and sample solutions were applied to the plates as 8 mm bands, 13 mm apart from each other and the Plate dimension 20 X 10 cm and 10 mm from the bottom edge of the plate, under a continuous spray of inert gas by means of a Camag Linomat V TLC sample applicator with a 100 µL syringe (Hamilton, Bonaduz, Switzerland). After the application, prederivatization was performed by exposing the plate to iodine vapor for 10 minutes. The prederivatized plate was developed vertically ascending in a twin-trough glass chamber (Camag, Switzerland) saturated with a mobile phase comprising petroleum ether: ethyl acetate: acetonitrile (8.2: 1.2: 0.1 v/v/v) (Stahl, 1969; Ghosh *et al.*, 1985; Kapadia *et al.*, 1985). The optimized chamber saturation time for the mobile phase was 20 minutes at room temperature. The chromatographic run length was 90 mm from the bottom edge of the plate. After development, the plate was air dried and derivatized by dipping the developed plate in anisaldehyde-sulphuric acid reagent for 2 seconds (Stahl, 1969; Ghosh *et al.*, 1985; Martelanc *et al.*, 2009). The plate was then air-dried for complete removal of anisaldehyde-sulphuric acid and heated at 110°C for 3 minutes. Densitometric scanning was then performed at = 580 nm for alpha-amyrin using Camag TLC scanner 4 with winCATS software version 1.4.6. The slit dimension used was 5.00 × 0.45 mm (micro) with a scanning speed of 20 mm/sec, throughout the analysis (Stahl, 1969; Martelanc *et al.*, 2007; Kpoviessi *et al.*, 2008). The applied chromatographic conditions permitted a well separation of the triterpenoid -alpha amyrin from the plant extract of two samples without any decomposition of alpha amyrin.

Linearity

Determination of linear dynamic range concentration of alpha amyrin was done by applying 5µL and 6 µL of TLC plate of working standard containing alpha amyrin. The peak area obtained from densitogram for each applied concentration of alpha amyrin was noted. The calibration curves of the standard were obtained by plotting graphs of the mean peak area of the standard versus the corresponding concentration.

System Suitability

The chromatographic separation was performed by injecting 5µL and 6 µL standard solution of alpha amyrin on a TLC plate in two replicates under specified chromatographic conditions (Table-1). The values of percent relative standard deviations of peak area from the chromatogram and retention factor of standards were taken as an indicator of system suitability. The value of the retention factor for standard alpha-amyrin was 0.49. As the values of percent relative standard deviation of peak area were found to be less than 2

and peaks were well-resolved, the method was considered worthy for analysis.

Specificity

The specificity of the HPTLC method was ascertained by comparing visible chromatograms of alpha-amyrin standard with the chromatogram of two samples. The chromatograms were compared by the overlay. A good correlation was observed between chromatograms obtained from standard and samples (Figure 1).

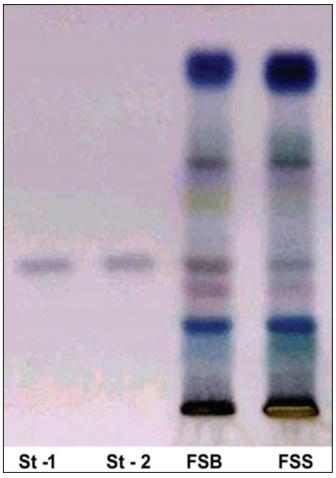

Figure 1: Represents the TLC plate [St-1; Standard 1: St-2; Standard 2: FSB-Sample of Bankiput: FSS-Sample of Sankarpur]

Assay

The developed and validated HPTLC method was used for quantification of alpha-amyrin from the extract of dried whole plant powder of *S. maritima*. 20 µL of each extract of plant materials of two zones was applied on the same TLC plate. The plate was developed and scanned under the specified chromatographic conditions.

Estimation of Alpha Amyrin

The amounts of alpha-amyrin present in each sample solution were determined from the calibration curve, by using the peak areas of alpha-amyrin in the sample. The flow rate of 150 nL/s was used.

RESULT AND DISCUSSION

Morphology

The general account of the annual herbaceous *S. maritima*, showed it to be quite bushy in nature with profuse branches (Figure 2A-C) and erect stem; stem reddish purple, glabrous, tender at younger parts and woody in the older region, internodes solid; leaves simple, alternate, sessile, exstipulate, linear, fleshy, semiterete, succulent and entire (Figure 2D). The inflorescence is a terminal spike and spike at leaf axil; flower is minute, ebracteate, bisexual, complete, regular, sessile, hypogynous, whitish green (Figure 2D); perianth 5, polytepalous, short,

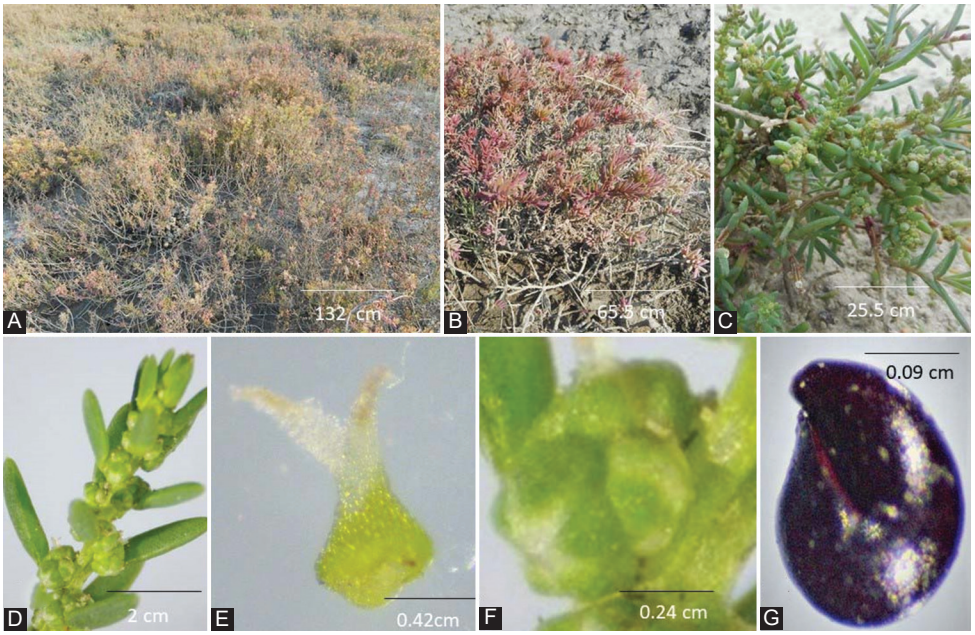

Figure 2: A-C *S. maritima* plant; D-Flowering twig; E-Carpel; F-Fruit; G-Seed

Table 1: HPTLC performance of standard α amyrin

Track	Sample	Applied volume	Start Rf	Start Height	Max Rf	Max Height	End Height	Area	Area %	Amount (mg/gm)	Sample ID
1	Standard	5µl	0.46	9.7	0.51	153.3	0.8	4197.9	48.55	0.05	Alpha amyrin
2	Standard	6µl	0.44	11.3	0.49	177.8	6.3	5079.6	50.38	0.06	Alpha amyrin

Table 2: Morphological traits of two population of *S. maritima*

Morphological characters \ Regions	FSB	FSS
Plant height (mm)	583±10.21	862±11.64
Stem circumference (mm)	44±2.37	62±2.41
Length of internode (mm)	83±3.89	118±5.2
Leaf length (mm)	19±1.58	25±2.1
Leaf breadth (mm)	2±0.17	3±0.33
Leaf thickness (mm)	0.8±0.07	1±0.13
Flower length (mm)	1±0.27	1.5±0.36
Tepal length (mm)	0.7±0.05	0.9±0.06
Fruit length (mm)	1.3±0.38	1.5±0.47
Fruit breadth (mm)	0.9±0.	1±0.16

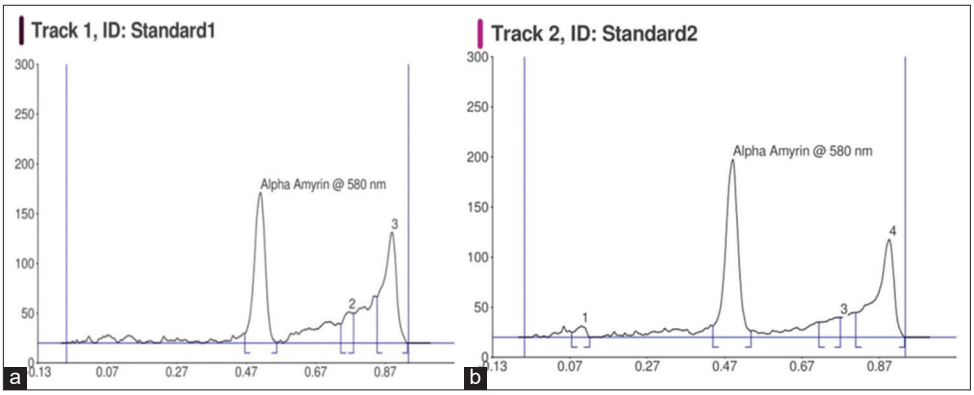

Figure 3: Represent HPTLC chromatogram of standard obtained at = 580 nm

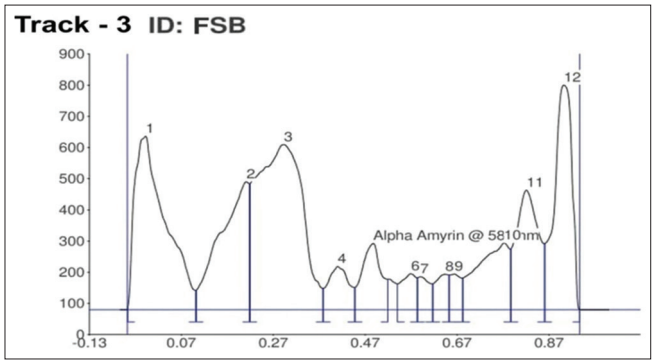

Figure 4: Represents HPTLC chromatogram of sample *S. maritima* at Bankiput obtained at = 580 nm

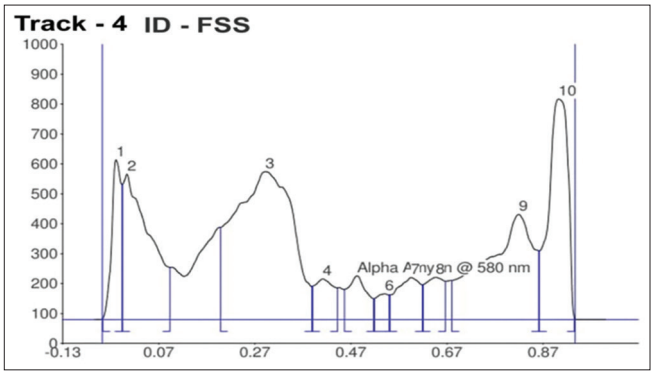

Figure 5: Represents HPTLC chromatogram of sample *S. maritima* at Sankarpur obtained at = 580 nm

globose or urceolate; stamens 5, free, filaments short, white, anther rather large, basifixed, bilobed; carpels 3, syncarpous, ovary superior, sessile, wide based, adnate below to perianth, ovoid, rounded apex, style minute, stigma 2, recurved (Figure 2E); fruit small, utricle with persistent perianth (Figure 2F); seeds with coriaceous testa and slender embryo (Figure 2G).

Variation in Morphology

Plants collected from two zones did not show any striking morphological variations though subtle variations plant height, stem circumference, leaf length, breadth, thickness, flower and tepal length and fruit length and breadth were noted (Table 2).

Amount of Alpha Amyrin

The confirmation of the method of measuring the amount of alpha amyrin with respect to standard has been displayed in the Table 1 and Figure 3. The amount of amyrin has been found to be greater in the plants of Bankiput (0.12 mg/g i.e. 4.37%) than those of Sankarpur (0.068 mg/g i.e. 3.123%) (Table 3, Figure 4 & 5).

CONCLUSION

The occurrence of subtle morphological changes along with the considerable difference in the amount of alpha amyrin

Table 3: HPTLC performance of α amyrin from two populations of *S. maritima*

Track	Sample	Applied volume	Start Rf	Start Height	Max Rf	Max Height	End height	Area	Area %	Alpha amyrin mg/gm
3	FSB	20µl	0.45	72.1	0.49	213.3	98.4	7752.0	4.37	0.12
4	FSS	20µl	0.45	100.9	0.48	146.6	70.6	5167.6	3.13	0.068

content among the individuals of *Suaeda maritima* growing at two distantly placed sites along the coastal area of West Bengal represents intraspecific diversity of it. Though the sites of their occurrence are contiguous and the individuals of the species are growing interruptedly in patches, the diversity, as witnessed, might be due to the difference in micro-environmental factors rather than the difference in their genetic content.

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