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ORIGINAL ARTICLE

Study of Estimation and Variation of Alpha-amyrin Content among individuals of *Suaeda maritima* (L.) Dumort. Growing along the South-East Coast of India

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Suaeda maritima (L.) Dumort. of the family Chenopodiaceae, is an annual succulent herb growing on salty marshy habitat as one of the dominant mangrove associate species and also as pure vegetation of that. It is regularly harnessed by the local people for use as food as well as for alleviating different maladies. Alpha-amyrin, a triterpenoid, is a remarkable biomolecule available in *S. maritima*. It is reported to have cardioprotective, anti-inflammatory and anti-oxidant properties. A survey of the amount of alpha-amyrin content available in the individuals of *S. maritima,* collected from eight different regions of the sea coast of the bay of Bengal like, Digha, Sankarpur, Tajpur, Dadanpatrabarh, Shoula, Bankiput and Petuaghat, was conducted with normal phase high performance thin layer chromatography (HPTLC) in this study. It also portrayed variation in the amount of alpha-amyrin among the plant individuals of the species growing in the said zones. The existence of variation in the amount of alpha-amyrin seems to be prospective for selecting the best producer out of them.

Key words: Alpha-amyrin, HPTLC, Suaeda maritima

Alpha-amyrin, a pentacyclic triterpenoid of medicinal importance has been reported to occur in different mangrove plants and mangrove associate species in earlier literatures (Pant and Rastogr, 1979; Ghosh *et al*., 1985; Ferreira, 2022). This active biomolecule possesses a wide spectrum of pharmaceutical and biological functions like, anti-microbial, insecticidal (Ekalu *et al*., 2019; Viet *et al*., 2021; Oliveria, 2022), anti-oxidant, anti-arthritic, anti-inflamatory, anti-nociceptive, anti-depressant, anti-hyperglycemic (Oliveira *et al*., 2005; Pinto *et al*., 2007; Holanda *et al*., 2008; Barros *et al*., 2011; Melo *et al*., 2011; Santos *et al*., 2012; Aragao *et al*., 2015; Beulah, 2021; Lakhmi, 2023), and anti-ulcer, gastroprotective properties (Oliveira *et al*., 2004; Prabhakar *et al*., 2017).

Suaeda maritima (L.) Dumort., the annual sea-blite, a herb of the family Chenopodiaceae is locally known as 'Giria Shak' in West Bengal. It is used by the village folks as vegetable and also for curing maladies of various nature (Trease and Evans, 2002; Singh *et al*., 2013; Han and Bakovic, 2015; Roy *et al*., 2021).

This species is generally found to grow along the coastal belts of Indian subcontinent as well as grow luxuriantly in the coastal belt of Purba Medinipur district of West Bengal in India (Das *et al*., 2015) and the present authors published a report (Pati and Nandi, 2022) on the morphological and phytochemical accounts of the species growing in the coast of Bay of Bengal in Purba Medinipur district.

Estimation of the quantity of alpha-amyrin among different individuals of this species, collected from distantly placed sites of the studied area, were carried out under this program in search of variation in the content, if any, among the individuals of different sites. The biomolecule having medicinal importance any variation in the amount thereof was expected to be worthwhile for sorting out the best producer from amongst the locally growing individuals of the species.

MATERIALS AND METHODS

Collection and identification of plant material

Plant samples were collected at their flowering and fruiting stage from eight regions of the cost of the district Purba Medinipur, namely, Digha (latitude-21^{0}63´08´´N & longitude-87^{0}54´63´´E), Sankarpur (latitude-21^{0}63´65´´N & longitude-87^{0}56´98´´E), Tajpur (latitude-21^{0}64´75´´N & longitude-87^{0}61´32´´E), Dadanpatrabarh (latitude- 21^{0}67´69´´N & longitude- 87^{0}71´86´´E), Shoula (latitude-21^{0}68´56´´N & longitude- 87^{0}76´07´´E), Junput (latitude-21^{0}72´09´´N & longitude-87^{0}82´07´´E), Bankiput (latitude-21^{0}76´52´´N & longitude- 87^{0}86´89´´E) and Petuaghat (latitude-21^{0}79´43´´N & longitude-87^{0}89´55´´E) which were situated 8-9 km apart along the coastal belt of Purba Medinipur district. Identity of the plant species was authenticated by the Central National Herbarium, Sibpur, India (vide – voucher specimen number CNH//2015/Tech.II/17/299). The voucher specimens were deposited at the Herbarium of Vidyasagar University, India.

Preparation of Sample Solution

The well cleaned aerial parts of the plant materials were chopped into pieces and dried in shade at room temperature and was pounded to powder in mechanical grinder. Twenty grams of the dried aerial parts of different groups of individuals taken as different samples were weighed and poured in 150 mL of methanol solvent. The mixture was stirred on every 30 minutes for 3 hour and thereafter kept as such for two days. The plant extracts were filtered separately using Whatman No 1 filter paper at room temperature. The obtained extracts were concentrated to one third of its original volume by rotary evaporator. The concentrates were dissolved in 2 mL of solvent of methanol: chloroform (1: 1, v/v) in a 10 mL volumetric flask and stored in a refrigerator for HPTLC analysis. Thereafter 0.50 mL of this solution was diluted up to 10 mL by methanol to obtain working standard solution of alpha-amyrin (Stahl, 1969).

Preparation of Standard solution

Standard alpha-amyrin (purity 99.3%), from Sigma-Aldrich Chemie GmbH (Aldrich Division, Steinbeim, Germany) was used as reference. Ten mg of alpha-amyrin was accurately weighed and transferred to 10 mL volumetric flask. Five mL of methanol solution was added into the volumetric flask and sonicated in an ultrasonic bath at a frequency 50 Hz for 5 minutes for complete dissolution of alpha-amyrin. The volume was

then made up to 10 mL with methanol. Thus, a stock solution of this standard chemical 1000 µg/mL was prepared (Martelanc *et al*., 2009).

Preparation of mobile phase

The mobile phase used in the present analysis was prepared by mixing petroleum ether, ethyl acetate, and acetonitrile in the volume ratio of 8.2: 1.2: 0.1 (v/v/v). During development of each plate, a fresh mobile phase was prepared.

High Performance Liquid Chromatography

Chromatography was carried out on 20 cm × 10 cm TLC aluminum pre-coated silica gel 60F_{254} plate, with 200 µm layer thickness (E. Merck, Mumbai, India). Standard and sample solutions were applied on the plates as 8 mm bands, 13 mm apart from each other and 10 mm from bottom edge of the plate, under a continuous spray of inert gas by means of a Camag Linomat 5 TLC sample applicator with a 100µL syringe (Hamilton, Bonaduz, Switzerland). After the application, pre-derivatization was performed by exposing the plate to iodine vapour for 10 minutes. The pre-derivatized plate was developed vertically ascending in a twin-trough glass chamber (Camag, Switzerland) saturated with mobile phase and the optimized chamber saturation time for the mobile phase was 20 minutes at room temperature. After development, the plate was air dried and derivatized by dipping in anisaldehyde-sulphuric acid reagent for 2 seconds. The plate was then air-dried for complete removal of anisaldehyde-sulphuric acid and heated at 110°C for 3 minutes. The slit dimension used was 5.00 × 0.45 mm with scanning speed of 20 mm/sec, throughout the analysis and the flow rate of 150 nL/s was used (Martelanc *et al*., 2009). Densitometric scanning was then performed at 580 nm for alpha-amyrin using Camag TLC scanner 4 with winCATS software version 1.4.6.

The applied chromatographic conditions permitted a well separation of alpha-amyrin from the plant extract of 8 samples without any decomposition of alpha-amyrin.

Linearity

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

System suitability

The chromatographic separation was performed by injecting 5µL and 6µL standard solution of alpha-amyrin on 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.

Specificity

The specificity of the HPTLC method was ascertained by comparing visible chromatograms of alpha-amyrin standard (Figure 1A & B) with the chromatogram of eight samples (Figure 2-6). The chromatograms were compared by overlay. Good correlation was observed between chromatograms obtained from standard and samples.

Assay

The developed and validated HPTLC method was used to quantify the amount of alpha-amyrin from the extract of dried whole plant powder of *S. maritima*. Twenty microliters (20µL) of each extract of plant materials of 8 zones was applied on the same TLC plate. The plate was developed and scanned under the specified chromatographic conditions (Figure 7). Identity of the bands of alpha-amyrin in the sample solutions was confirmed by comparing the value of retention factor in samples with that of the reference standard. The value of retention factor (Rf) for alpha-amyrin was 0.49.

Estimation of Alpha-amyrin

The amount of alpha-amyrin present in each sample solution was determined from the calibration curve, by using the peak areas of alpha-amyrin in the sample.

RESULTS

The results of assay of alpha-amyrin from the plant samples of 8 places are displayed in the Table 2. The typical HPTLC chromatogram of standard alpha-amyrin is shown in the Figure 1A & B, while the chromatograms of the extracts from dried whole plants of eight

populations of *Suaeda maritima* have been presented in the Figure 2-6. In the study of HPTLC analysis for a qualitative determination of alpha-amyrin and evaluation of their presence in plant extracts *S. maritima*, the retention factor (Rf) value for alpha-amyrin was 0.49.

The amount of alpha-amyrin was found to vary from 0.03 mg/g to 0.22 mg/g in the populations of the plant species *S. maritima* growing in different patches along the coastal area of Purba Medinipur district of West Bengal in India (Table 2). The greater amount of alpha-amyrin was found to occur in the plants growing at Digha, Tajpur, Shoula and Petuaghat regions. The highest amount was noticed in the plants from Digha (Figure 3), shown in TLC plate as track DG (Figure 7) and the lowest in the community from Dadanpatrabarh (Figure 4), shown in TLC plate as Track DP (Figure 7).

Table 1. HPTLC performance of Standard alpha-amyrin.

Sample	Applied volume	Start Rf	Max Rf	End Rf	Area	Amount (mg/g)
Standard (S1)	5 µL	0.46	0.51	0.56	4197.9	0.05
Standard (S2)	6 µL	0.44	0.49	0.54	5079.6	0.06

Table 2. HPTLC performance of alpha-amyrin from eight populations of *S. maritima*.

Sample No	Sample	Applied volume	Start Rf	Max Rf	End Rf	Area	Amount (mg/g)
1.	DG	20 µL	0.47	0.51	0.54	12397.1	0.22
2.	SN	20 µL	0.45	0.48	0.52	5167.6	0.06
3.	TJ	20 µL	0.46	0.49	0.54	8972.9	0.15
4.	DP	20 µL	0.48	0.51	0.55	3750.7	0.03
5.	SH	20 µL	0.45	0.48	0.52	8731.4	0.14
6.	JN	20 µL	0.49	0.52	0.56	4399.3	0.05
7.	BN	20 µL	0.45	0.48	0.51	5817.2	0.08
8.	PG	20 µL	0.44	0.48	0.51	7309.5	0.11

[DG- Sample of Digha; SN- Sankarpur; TJ- Tajpur; DP- Dadanpatrabarh; SH- Shoula; JN- Junput; BN- Bankiput; PG- Petuaghat].

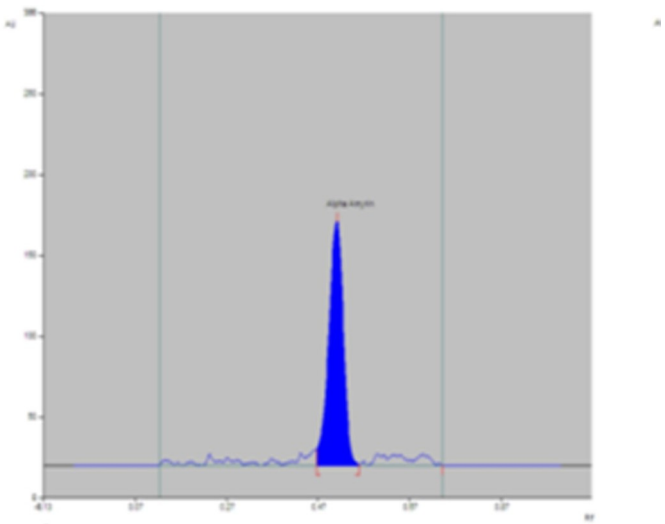

Figure 1. HPTLC chromatogram of standard of alpha-amyrin.

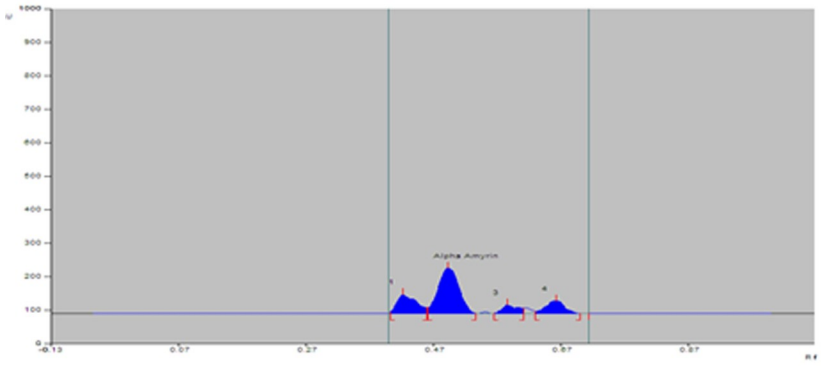

Figure 2. HPTLC chromatogram of sample of *S. maritima* from Tajpur (TJ).

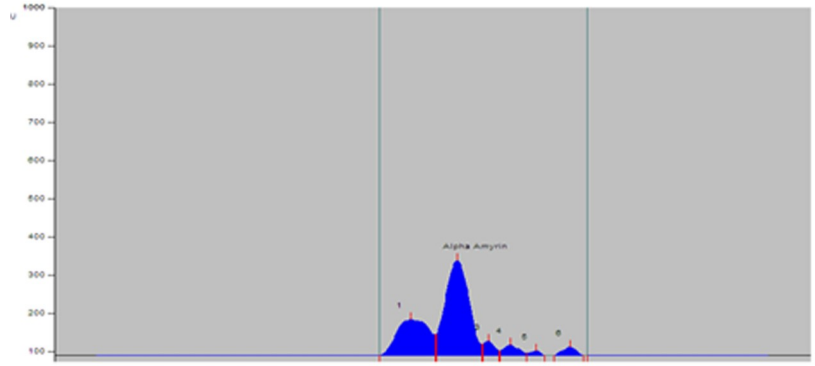

Figure 3. HPTLC chromatogram of sample of *S. maritima* from Digha (DG).

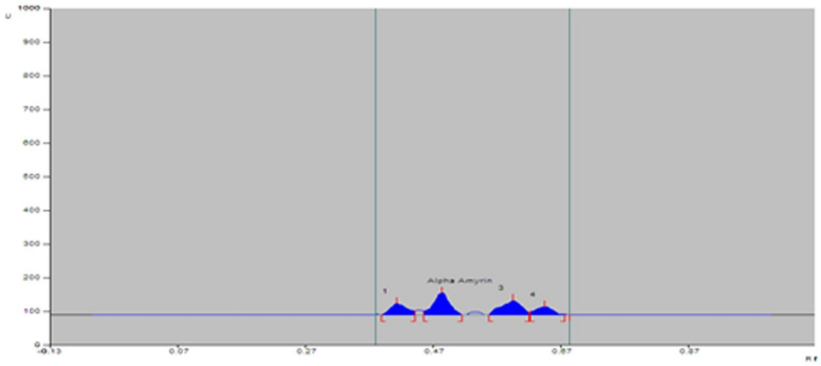

Figure 4. HPTLC chromatogram of sample of *S. maritima* at Dadanpatrabarh (DP).

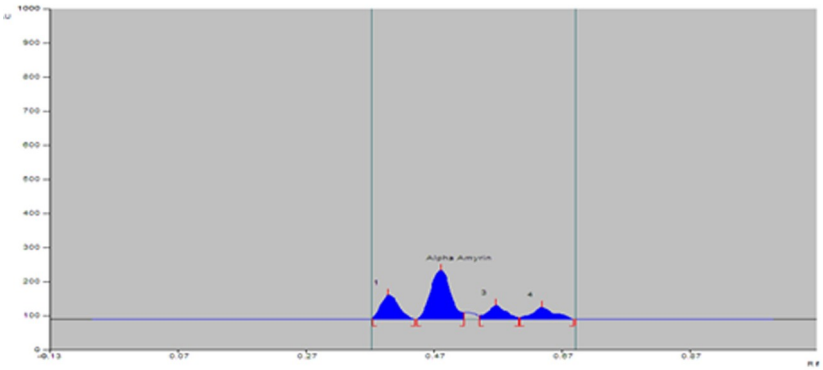

Figure 5. HPTLC chromatogram of sample of *S. maritima* from Petuaghat (PG).

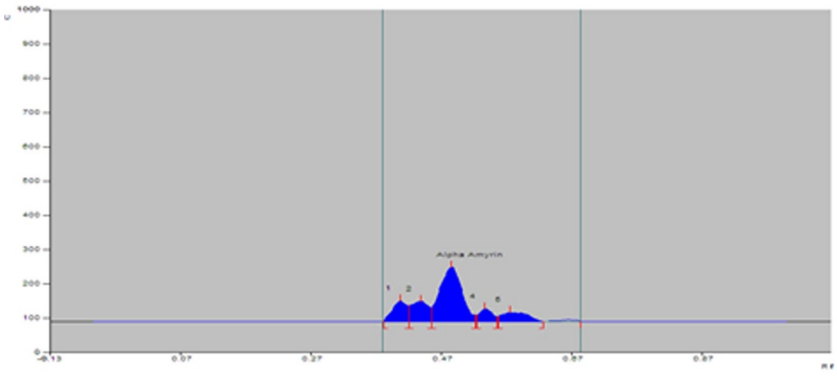

Figure 6. HPTLC chromatogram of sample of *S. maritima* from Shoula (SH).

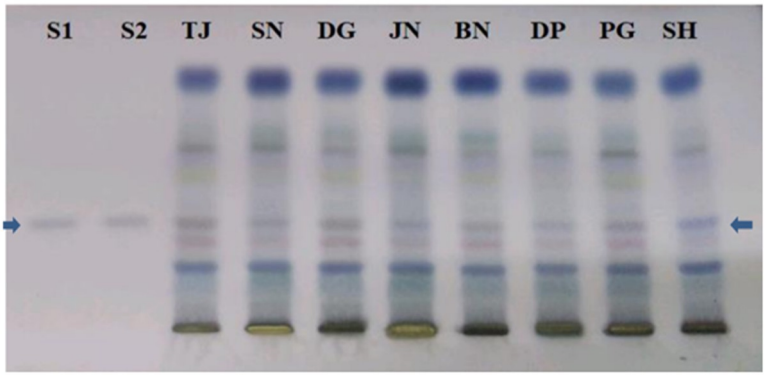

Figure 7. TLC of alpha-amyrin (band marked with arrows) of the standards and from the samples of *S. maritima* from different sites [S1-Standard; S2- Standard; DG- Digha; SN- Sankarpur; TJ- Tajpur; DP- Dadanpatrabarh; SH- Shoula; JN- Junput; BN- Bankiput; PG- Petuaghat].

DISCUSSION

The study showed considerable difference in the amount of alpha-amyrin content among the individuals collected from different spots of the studied area. However, the cause of such variation can hardly be attributed to their genetic difference, as even the plants being grown at different locations situated far apart from one another, they grow quite contiguously and under the regular influence of tidal waves, which always disseminate the seed propagules indiscriminately leaving least scope for growing of populations with different genetic makeups at different sites.

Earlier works on different plants have shown that the physical factors like heat (Gill and Tuteja, 2010; Pucciariello *et al*., 2012; Fortunato, 2023) or salt stress (Hossain and Dietz, 2016; Yang and Guo, 2018; Behera, 2021; Zang, 2021; Yu B, 2022; Mondal *et al*., 2023) can lead to the production of greater amount of reactive oxygen species and also the increased production of the biochemicals like, proline, glycine betaine, a host of secondary metabolites including amyrins, to maintain the osmotic balance, to combat the ROS (Reactive oxygen species) and damage due to salt stress. Choi *et al*. (2012) recorded higher proline content in *S. maritima*, as a measure to control salt stress, a feature which also relate to the occurrence of amyrin along with other phenolic contents in the plant. Changes in biomolecules under the influence of salinity has been documented in different experiments, as the activity of Superoxide dismutase was recorded to get increased (Wang, 2004; Guan *et al*., 2011; Sachdev, 2021) and the gene for Glutathione transferase was found to be upregulated (Sahu and Shaw, 2009) in *S. maritima* tissues with the increase of salinity. So, the changes in metabolite content have been claimed (Yu *et al*., 2022) to play vital role in maintaining cell osmotic potential, cell membrane structure, as well as in destructing ROS. In consideration of these facts and Digha being situated on

the bank of the ocean, Bay of Bengal, in comparison to other sites, taken in the survey, and consequently having greater concentration of salt, the occurrence of greater amount of alpha-amyrin in the plants growing at this site seems to be quite up to the expectation. So, the difference in the amount of alpha-amyrin recorded in the studied populations of *S. maritima* is least likely due to any genetic difference between them, instead, due to the difference in micro-environmental factors the plants are subjected to.

CONCLUSION

The present work expressed the potentiality of *S. maritima* as a source of the medicinally important compound alpha amyrin by HPTLC. Difference in the amount of alpha amyrin content recorded amongst the individuals of *Suaeda maritima* growing in the studied area represents intraspecific diversity in respect of that medicinally important biomolecule. The difference in the amount of alpha-amyrin among the contiguously growing individuals might be due to the difference in micro-environmental factors of the growing sites rather than the difference in their genetic content. A further in-depth study may confirm the reason behind it. Regular intake of it as food by the local people is expected to be beneficial for them. Proper exploitation of the best producer individuals by pharmaceutical industries may be useful for human welfare in a bigger way.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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