SINCE 2010

NAAS Rating
2012:1.3; 2013-16: 2.69
2017-2020: 3.98

IMPACT FACTOR
2019-20: 2.40; 2021:1.09

IPI Value
1.92

Received on:
10th May 2021
Revised on:
24th June 2021
Accepted on:
26th June 2021
Published on:
1st July 2021

Volume No.
Online & Print
137 (2021)

Page No.
10 to 17

Life Sciences Leaflets is an international open access print & e journal, peer reviewed, worldwide abstract listed, published every month with ISSN, RNI Free- membership, downloads and access.

A REVIEW ON *SALICORNIA BRACHIATA* (ROXB.) AS A POTENTIAL DIETARY SUPPLEMENT

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ABSTRACT:

Salicornia brachiata Roxb. is a member of the Amaranthaceae family, and is an extreme halophyte that is a potential marine agricultural alternative crop. Due to its potential as a naturally adapted higher plant model for abiotic stress-responsive gene resources, this halophyte is a promising alternative with a variety of uses. *S.brachiata* is a promising candidate with a wide range of nutritional qualities and uses. Oligosaccharides were discovered after analyzing a wide spectrum of carbohydrates and carbohydrate-containing substances. The oil content was Analysed using GC-MS. Proteome profiling study was carried out using MALDI TOF-TOF MS in protein rich seeds of *S.brachiata*. Sulphur-rich proteins with special oligosaccharides were found in *S. brachiata*, and the strategy for enhancing the nutritional quality of seed proteins has the potential to be applied on a larger scale, but further research and knowledge are required. All of the extraction and identification were then matched to a commonly utilized nutrition source. All of the extraction and identification were then matched to a commonly utilized nutrition source.

KEYWORDS: *Halophytic plant, Dietary supplement, OLIMP, GC-MS, MALDI-TOF MS, Nutritional potential.*

Salicornia brachiata Roxb. (Amaranthaceae) is an extreme halophyte considered as a potential substitute crop for seawater agriculture (Jha et

al., 2012). It is an annual high salt- tolerant plant with green, joined, leafless, succulent stems (phylloclade) and terminally fruit- bearing spikes (Mishra et al., 2012).

S. brachiata grows opulently in salt marshes; it is cultivated in areas with elevated salinity (Glenn *et al.*, 1999; Stanley, 2008). This halophytic plant is having the capabilities of accumulating 30-40% of the dry weight of NaCl during in-vitro regeneration (Glenn *et al.*,1999; Joshi, Mishra & Jha 2012). This halophyte is a promising candidate with a variety of applications due to its potential as a naturally adapted higher plant model for abiotic stress responding gene resources (Chaturvedi, Mishra, Tiwari, & Jha, 2012; Jha, Sharma, & Mishra, 2011). Protein-rich seeds, as well as shoots of the extreme halophyte S. brachiata, are eaten as salad greens by considering their quality nutritional value (Jha, Singh, & Mishra,2012).

S. brachiata is a promising candidate having various nutritional properties, with a range of applications (Mishra *et al.*, 2012). The study was focused on Oil analysis carried out using GC–MS by hexane and petroleum ether extraction by Eganathan*et al.*, 2006). Oligo- saccharide mass profiling (OLIMP)(Mishra *et al.*,2012) and proteome profiling study was carried out using MALDI TOF-TOF MS (Jha *et al.*, 2012).

This scientific investigation looked at the oil content of Salicornia brachiata seeds. Seeds with the highest oil content were extracted with hexane (22.4%). The oil's high ester (538.32 mg/g) and saponification (547.52 mg/g) levels indicate that it could be used in industry.

The yields of seed oil extracted from *S. brachiata* using two solvent systems, petroleum ether, and hexane, when the seed was extracted with petroleum ether 10.5% whilemaximum oil yieldcan be extracted by hexane method.

By comparing *Salicornia* seed oil to other edible oils, unsaturation (a measure of iodine value) is very low, with the exception of coconut oil. If the double bonds are not conjugated with each other or with carbonyl oxygen, the iodine value can be used to calculate unsaturation (Allen, 1955). *S. brachiata* has a very high saponification value (547.5), which indicates the average molecular weight of fat. Despite the fact that the total percentage of oil in Salicornia seeds is 22 %, the oil is unlikely to be edible due to the high content of saponins in the seeds. However, by carefully washing the seeds or flour in running water, saponfrom the seeds or flour will be leached out, making it more edible. Since a high ester value indicates a high volume of glycerides, it can be used as a soap and detergent substitute. According to Glenn *et al.*, 1998 *S. bigelloviihad* a high saponification value as well. The seed oil content and composition of *S. brachiata* have yet to be investigated. Our ongoing studies into the oil yield and utility of this species' seeds may aid in the commercialization of the species in areas where it can thrive.

Carbohydrates are one of the most common macromolecules in nature and have a wide range of structures. Based on the linkage of constituent monosaccharide residues, oligo and polysaccharides seem to be either linear (proteins and nucleic acids) or branched. Naturally occurring carbohydrates, such as dextran, cellulose, and glycogen, are made up of several units of monosaccharide residues that are organized in distinct patterns like linear, branching, or both (linear & branching), and have a unique structure. By glycosylation process, saccharides or glycans increase the complexity of proteins, by influencing protein folding, modulating protein structure, and facilitating the identification of binding ligands (Zaia, 2004). Saccharides are essential in food and nutritional chemistry, biofuels, biomaterials, and pharmaceuticals, in addition to their basic functions at the cellular and biomolecular levels.

Carbohydrate polymers serve as structural and storage molecules in plants. For mass spectrometric analysis, these are massive macromolecules that must be degraded enzymatically or chemically. Oligosaccharide mass profiling (OLIMP) in combination with matrix-assisted laser desorption ionization-time of flight-time of flight (MALDI TOF-TOF) mass spectrometry technique is a convenient, fast, sensitive, and accurate for analyzing a wide range of carbohydrates and carbohydrate-containing compounds (Harvey, 1999; Zaia, 2004; Persson, Sørensen, Moller, Willats, & Pauly, 2010). The technique is salt-independent and does not need derivatization, which is necessary by most traditional mass spectrometric techniques. With a limited amount of samples, it can be used to study large mass molecules and a wide diversity of organisms (Günl, Kraemer, & Pauly, 2011; Mishra, Kavita, & Jha, 2011; Obel, Erben, Schwarz, Kühnel, Fodor, & Pauly, 2009; Ropartz*et al*., 2011; Westphal, Schols, Voragen, & Gruppen, 2010). Moreover, OLIMP is also a preferred modern versatile analytical tool with high-performance efficiency that can be used in situ with tissues or seedlings (Obel*et al*., 2009; Günl*et al.*, 2011).

Mishra *et al.*, (2011) had observed MALDI TOF-TOF mass spectroscopy was previously designed for extracellular polysaccharides, and only positive ion mode fragmentation peaks for oligosaccharide and polysaccharide analysis Positive ion linear and reflector modes were found to be optimal, respectively, whereas negative ion linear and reflector modes did not detect fragmentation peaks. The current study reflects of attributable to monosaccharide units and derivatives depending upon range of mass peaks (Ropartz*et al*., 2011).

The plant cell wall is made up of cellulose, hemicellulose, pectin, lignin, and other substances. OLIMP is a semi-quantitative fingerprinting method that relies on the digestion of cell wall polymers (Obel*et al.*, 2009), which showed that the cell wall of *S. brachiata* is made up of a series of monomers. A similar profile was previously reported in Arabidopsis cell walls (Westphal *et al.*, 2010). The hemicellulose xyloglucan (XXXG, X [XL]G, XXFG, and XLFG) is a component of

vascular dicot cell walls that plays a key role in the cell wall structure and function. The presence of xyloglucans in the cell wall of *S. brachiata* was discovered using oligosaccharide mass profiling. The supremacy of XXXG over other xyloglucans was revealed by MS spectral analysis. In the cell walls of highly nutritious plant soybean (Huisman, Weel, Schols, &Voragen, 2000) and model plant tobacco (Nguema-Ona et al., 2012), the characteristic mass peak (m/z) of poly XXXG xyloglucan oligomers (XXG, XXXG, XXFG, XLXG, and XLFG) was identified. Despite the fact that mass spectroscopy cannot differentiate diastereomers, it may reveal the number and type(s) of sugar moieties present in an oligomer (Mishra et al.,2011).

Halophyte *S. brachiata* is being considered as a possible substitute crop for seawater agriculture. Its OLIMP showed the typical mass peaks (m/z) of mono- and oligosaccharides, as well as their derivatives. The mass profile showed that XXXG was more dominant than other oligosaccharides, and thus resembled the mass profile of a highly nutritive soybean plant. The nutritional potential of this plant as a latent source of dietary supplementation is revealed in this report. This is the first time oligosaccharide mass profiling (OLIMP) has been done on any edible halophyte. To obtain detailed structural information about oligomers, further linkage analysis is needed.

In the context of the dietary use of *S. brachiata* (Amaranthaceae), an essential plant with a broad range of adaptation, the study focuses on seed protein proteome profiling. This is the first analysis that came to the knowledge of the polypeptide subunit composition of the globulin fraction of seed proteins from *S. brachiata* that confirms the existence of inter and intra-molecular disulfide linkages in the major fraction of SSP. It was the first research to demonstrate the existence of inter and intra-molecular disulfide linkages in the major fraction of SSP in the polypeptide subunit composition of the globul infraction of seed proteins from *S. brachiata*. Seed protein was determined by mechanically grinding seeds, which were then processed into seed meal, which contained seed storage proteins (e.g., glutelins, globulins, albumins, prolamins). Sequential extraction with various solvents was used to fractionate these SSPs (Jha et al., 2012).

According to Bhavnathjha Protein concentration was estimated using the Bradford method and expressed in terms of dry weight using a standard curve of bovine serum albumin (BSA). The collected fraction was mixed 1:1 with sample buffer (0.2 mM Tris-HCl buffer (pH 6.8), 2% SDS, 10% glycerol, and 0.025% bromophenol blue) to make the samples. 1D 12% SDS-PAGE (gel scale 18 16 cm) was used to evaluate samples (15 g of each protein fraction). For 2D nonreduced/reduced SDS-PAGE study, the proteins (15 g) were first isolated under nonreducing conditions. The first-dimension gel strip was equilibrated in 0.2 mM Tris-HCl buffer (pH 6.8) with 2% SDS and 2% 2-mercaptoethanol before being loaded onto the second-dimension

polyacrylamide gel. Reproducible spots (common spots) were chosen for in-gel digestion and MALDI-TOF MS analysis.

Manually excised protein spots from CBB stained gels were processed for in-gel digestion and MALDI-TOF MS analysis. For 1 hour at 40 °C, each protein-containing gel slice was fully destained in 50 mM NH4HCO3 and 50 %v/v ACN. After that, the stained slices were incubated for 30 minutes at room temperature with iodoacetamide (55 mM) before being incubated for 15 minutes with acetonitrile (50 percent v/v). After that, the slices were lyophilized, rehydrated, and incubated overnight at 37 °C with sequencing grade adjusted trypsin (10 ng/L) in 25 mM NH4HCO3. Peptides were collected after digestion, and the gels were washed three times with 0.1% TFA in 50% ACN to collect the residual peptides. A MALDI-TOF mass spectrometer (AXIMA CFR plus, Shimadzu Biotech, Kyoto, Japan) in reflector mode was used to characterize the trypsin-digested peptides over a mass range of 700-3000 Da. The spectrum's reproducibility was tested using five-spot sets, and the spectra were analyzed after centroid and deisotoping. With carbamidomethyl (C) as a fixed modification and Gln→pyro-Glu (N-term Q) and Gln→pyro-Glu (N-term E) as variable modifications, a peptide mass tolerance of 0.5 Da, and one overall mixed cleavage, monoisotopic peaks of peptide mass fingerprints were obtained. The MASCOT database was used to scan the peptide mass fingerprint data for comparative proteinhomology.

Classification of Proteins: The UniProt database (http://www.ebi.uniprot.org/index) was used to check the listed proteins for their known functions. Using functional catalogue software, proteins were divided into groups based on their biochemical roles. Four seed protein fractions, including albumins, globulins, glutelins, and prolamins, were fractionated and analyzed for their relative protein proportions in seeds of *S. brachiata* based on solubility criteria.

Salt-soluble fraction globulins were found to be the most abundant of the four protein fractions (54.75 %), followed by water-soluble protein albumins (34.30 %) and alkali-soluble glutelins (8.70 %). The proportion of alcohol-soluble protein (prolamins) in total seed protein was the lowest (2.25 %). The pattern of fractionation was close to that of leguminous seed proteins.

SDS-PAGE was used to screen for constituent polypeptides in seed protein fractions under nonreducing and reducing conditions. Under both conditions, polypeptides with a molecular mass of 10124.4 kDa were found. Under both conditions, polypeptides with a molecular mass of 10124.4 kDa were found. Salt-soluble globulins with a significant number of polypeptides were resolved as intensity bands. Under nonreducing conditions, polypeptides of were resolved as low-intensity bands. Polypeptides were vanished under decreasing conditions, while new bands were appeared with high intensities.

The presence of disulfide bonds in the globulin fraction was revealed by polypeptide profiling under reducing and nonreducing conditions. Individual bands observed under nonreducing conditions were compared to those obtained under reducing conditions using two-dimensional polyacrylamide gel electrophoresis because the globulin fraction exhibited significant disulfide bonds. Polypeptides with disulfide bonds were quickly reduced and transferred as spots off the diagonal in a 2D gel with nonreducing conditions in the first dimension and reducing conditions in the second dimension. In the second major fraction of albumins, disulfide-linked polypeptides were not found. The breakage of intermolecular or intramolecular disulfide bonds by β-mercaptoethanol affected the electrophoretic mobility of proteins. In the second dimension, breaking intermolecular disulfide bonds between subunits resulted in different subunit polypeptides. Within a single polypeptide, cleavage of intramolecular disulfide bonds induced a conformational shift that increased or decreased mobility during SDS-PAGE. Unreactive proteins were limited to a diagonal by the diagonal SDS-PAGE used in this analysis, while proteins outside the diagonal had thiols near enough to form intra-molecular disulfide bonds. Seven intra-molecular disulfide-linked polypeptide pairs with acidic (large) and basic (small) subunits were discovered.

Salicornia globulin seed protein purified globulins Matrix-assisted laser desorption/ionization mass spectrometry was used to further examine from 2D SDS-PAGE (MALDI-TOF MS). Disulfide linkages were identified in 32 protein spots on the 2D-diagonal gel. Spots were removed and processed for trypsin digestion in the gel. MALDI-TOF MS was used to test trypsin-digested polypeptides, and the peptide mass fingerprint data was used to scan the UniProt database for functional recognition. Based on their biochemical properties, proteins were further divided into seven classes.

The proteins found in the study were linked to 13 different plant species*: Arabidopsis thaliana, Timmiamegapolitana, Oryza sativa, Triticum aestivum, Fragaria ananassa, Brassica compestris, Antirrhinum majus, Medicago truncatula, Gossypium herbasium, Allium cepa, Lycopersicon esculentum, Cyathea alata,* and*Zeamays.*

The existence of inter and intra-molecular disulfide bonds in globulins, the main fraction of seed storage proteins, is confirmed by peptide mass fingerprint analysis. Important amino acids are lacking in plant proteins, making them inferior to animal protein sources. *S. brachiata*, an intense halophyte that grows well in saline environments, is being considered as a possible substitute crop for seawater agriculture. The sulfur-rich proteins are thought to be very nutritious, and the presence of intermolecular and intramolecular disulfide bonds makes this plant a good source of dietary supplementation (Glenn et al.,1999).

When the gene encoding this protein is genetically engineered into target plants, it may compensate for the amino acid deficiencies of many seed proteins because of its high nutritional value. Sulfur-rich proteins may be ideal candidates for enhancing the nutritional quality of seed and vegetative tissue of plants intended for ruminant feeding in this way. Before this method for improving the nutritional quality of seed proteins can be used on a large scale, further study and understanding are needed.

Sulphur-rich proteins with special oligosaccharides were found in *S. brachiata*, and it was suggested that it could be used as a nutritive supplement. Furthermore, the current research discovered that Salicornia contains bioactive metabolites, indicating that the plant has medicinal potential for use as a functional food. Furthermore, its nutritional antioxidants, scavenging activities, amino acids, flavonoids, essential FAs, and PUFAs render it a promising candidate for use as a functional food, dietary supplement, and in the nutraceutical industry.

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