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Screening of Halophytic Oil Seed Species as a Potential Source of Protein and Oil Seed Crops

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ABSTRACT

A rapid decline in the fresh water supply and increasing salinization of agricultural land makes it imperative to seek alternative crop plants that could be grown in marginal lands with low quality water irrigation. *Suaeda fruticosa*, *Haloxylon stocksii* and *Cressa cretica* are highly salt-tolerant plants which are indigenous to the salts marshes and deserts of Pakistan. Current investigation explores the utility of these as oil seed crops. Biochemical analysis, including lipid composition, total proteins, carbohydrates and fractionation of neutral lipids, phospholipids and glycolipids of these halophytic oil seed species was performed. The total lipid content ranged from 3.27-11.83%. Total protein and carbohydrate content ranged from 19.0-33.0 and 5.50-15.0%, respectively. Fractionation by column chromatography showed the presence of neutral lipids, phospholipids and glycolipids. Thin layer chromatography for neutral lipids confirmed the presence of sterols, sterol-esters, mono-, diacyl- and triacylglycerols, and free fatty acid. Results described here indicate that these halophytic oil seed species could become an important oil seed crop for arid-land agriculture.

Keywords: *Cressa cretica*, halophytes, *Haloxylon stocksii*, salinity, *Suaeda fruticosa*

INTRODUCTION

Large parts of the world, arctic, Atlantic, semi-arid and arid climate zones, are naturally saline or have become salinated as a result of bad irrigation practices. Naturally saline soil occurs along the coastline of the continents and along borders of estuaries (salt marshes) and at inland saline zones (Rozema 1996). Arbitrarily saline soils have been described as soil with a soluble salt content exceeding 0.1% of the dry weight of the soil and reducing plant growth. More precisely, halophytic plants are assured to tolerate a salinity of 0.5% NaCl in soil water (or nutrient solution) (Gorhm 1995).

Halophytes are distributed in coastal and inland saline habitats throughout the world and their populations are subjected to high mortality risk because of the direct action of high salinity stress or other associated abiotic factors (Ungar 1991). Halophytes that primarily dominate subtropical deserts are shrubby perennials and most of the seeds of perennial halophytes have a hard seed coat that helps them to survive the harsh stressful conditions of high temperature and drought.

Consumption of edible oil in Pakistan and throughout the world is gradually increasing. Many alternatives are yet to be found for this purpose and therefore it has become imperative to find different non-conventional resources for the production of edible oil. One of the best means can be the use of halophytic species as the seeds of halophytic oil seed species are rich in oil content. In Pakistan cotton seeds are the major domestic source of edible oil followed by rape, mustard and canola (Anonymous 2005). Hu (1997) reported that saturated fats are more dangerous to health than poly-unsaturated fatty acids. It was reported earlier that palm oil and canola oil have 52 and 8% saturated fat, respectively while animal fats (lard) have 40% (Declercq and Daun 1998; Weber *et al*. 2001). Oil from *Salicornia bigelovilii* and another salt-tolerant perennial species *Suaeda moquinii* are reported to be of good quality compared with conventional oil (Glenn *et al*. 1991; Weber *et al*. 2001). This observation is further supported by reports on other halophytes such as *Crithmum maritimum*, *Zygophyllum album* (Zarrouk *et al*. 2003) and *Kosteletzkya virginica* (He *et al*. 2003).

In the current study the biochemical composition of seeds of three halophytic plants i.e. *Suaeda fruticosa*, *Haloxylon stocksii* and *Cressa cretica* were studied. These seeds were evaluated for their protein, carbohydrate and lipid contents with special reference to the composition of oil contents to be used as oil seed crops. *S. fruticosa*, a leaf succulent perennial with a woody stem in the family Chenopodiaceae is widely distributed in salt marshes and salt deserts of Karachi, Pakistan. *S. fruticosa* usually grows in warm, dry and high saline habitats and maintains a large seed bank in inland saline communities (Khan 1991). *H. stocksii* (Chenopodiaceae) is a leaf succulent perennial that is distributed from southern Sindh and Baluchistan up to the northern Himalayan mountain valley of Chitral, Pakistan (Stewart 1972) whereas *C. cretica* (Convolvulaceae) is a sub-erect dwarf shrub distributed widely in warmer and tropical regions of the world. It is usually found growing in pure stands or sometimes associated with other halophytes such as *Atriplex stocksii*, *S. fruticosa* and *H. stocksii*. *C. cretica* germinates in up to 860 mM or higher NaCl concentration (Khan 1991) and produces a large number of seeds (Khan and Aziz 1998).

MATERIALS AND METHODS

Chemicals and reagents used

All the chemicals and reagents used throughout this study were of analytical grade.

Sample acquisition

The seeds of *S. fruticosa*, *H. stocksii* and *C. cretica* plant species were acquainted locally and were collected during the months between April-July from salt flats of Karachi, Pakistan.

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Sample cleaning

The plants seed samples were first cleaned off from the trash metals, stick, stoves and weed seeds. The oil seeds were separated from their hulls by abrasion between the lined plastic sheets, in such a way that the seeds were not damaged.

Moisture content determination

The moisture content was estimated by the standard method of A.O.A.C. One gram seed sample was crushed in the mortar and pestle and was placed in a pre-weigh crucible. The sample was then dried in an oven at 105°C until a constant weight was attained. Moisture content was calculated as the difference between wet weight and dry weight of the sample and was recorded as gram/100 g tissue.

Estimation of ash content

One gram seeds of each plant was crushed and placed in a pre-weighted crucible. The crucibles were placed in the Muffle furnace at 500°C until a constant weight was attained. The ash content was calculated as the difference between the net weight and inorganic weight and recorded as g/100 g tissue.

Extraction of lipids

Solvent extraction method was used for the extraction of total lipid content (Bligh and Dyer 1959). Each sample (3.0 g) was crushed in mortar and pestle and was then homogenized for 10 min in 37.5 ml of chloroform: methanol: acetic acid mixture (100: 200: 75, v/v). The whole solvent mixture was then transferred in a separating funnel. Hundred milliliter distilled water was added and the system was blended again for 30 sec. The chloroform and the aqueous phase were allowed to phase separate for 2 h. After allowing the filtrate to separate into two layers the chloroform phase which contained the lipid was collected in a graduate cylinder. The lipid content was determined by weighing the sample after evaporation of the known aliquot of the chloroform to dryness at 50°C in a water bath. The lipid residues were dissolved in a small amount of chloroform and stored at −20°C for further analysis.

Estimation of total lipid

Photometric determination of total lipid was carried out by the method based on Phospho-vanillin method (Frings and Dunn 1970).

Estimation of triglyceride

Triglycerides were estimated by colorimetric enzymatic method as described by Bucolo and David (1973).

Fractionation of lipid

Total lipid extracted from the each seed was fractionated using silica gel adsorption chromatography with a solid stationary phase, Silica gel-60 (0.063-0.22 mm). Stepwise or batch elution was used to separate phospholipids, neutral lipids and glycolipids with methanol, chloroform and acetone, respectively.

Packing of column

The column was prepared by suspending 15.0 g silica gel-60 in 50 ml chloroform and immediately poured into a column of 30 × 2 cm size. Air bubbles, cracks and space were removed from the silica gel bed in order to make it homogenous.

Loading of sample

Total lipid extract (1.0 ml) was applied on top of the silica gel column with automatic applicator.

Elution of fractions

Neutral lipids, glycolipids and phospholipids were eluted with 100 ml of chloroform, acetone and methanol, respectively. Rate of elution for chloroform and acetone was adjusted with a speed of 0.5 ml/min while methanol as 0.25 ml/min.

Absorption

All the fractions were read on Shimadzu UV-160A spectrophotometer and the peak were determined on different wavelengths. Fraction having same wavelength were pooled together and concentrated in a water bath at 50°C. All the lipid fractions were re-solubilized in 0.5 ml chloroform for further studies.

Thin layer chromatography

Preparation of TLC plates

Glass plates (20 × 20 cm) were used for the preparation of TLC plates. Slurry of the sorbent was prepared by dissolving 26.0 g silica gel into 56.0 ml distilled water and mixed thoroughly; the slurry was poured into the Desaga Heidelberg's applicator to form a uniform thin layer of silica gel. Plates were air dried and kept for 45 min at 110°C for activation.

Sample loading

The samples and standard were loaded on to the plates with the help of capillary tube on one end of the plate (origin) and than air dried.

Development of plates

After application of samples and standards, the plate was transferred to the development chamber containing the solvent system; petroleum ether: diethylether: acetic acid (85: 15: 1; v/v). The level of the solvent in the chamber was adjusted in a way that it covers the absorbent up to a level of 5.0 mm from the bottom edge of the plate (Mangold 1966). The plate was developed by ascending chromatography for a specific time interval until the solvent front reached to a predetermined distance.

Visualization of spots

For initial visualization of the spots the air dried plate was placed in a tank saturated with iodine vapors. After appearance of dark yellow spots the plate was removed from the tank and the iodine was allowed to sublime and the spots were allowed to disappear.

Charging of plates

The plate was sprayed with 20% sulfuric acid and air dried after this plate was kept at 110°C for 5 min and the appearance of violet spots were marked and the plate was again placed at 110°C for 60 min for final spotting.

Estimation of protein

Total protein was estimated by Lowry's method using bovine serum albumin as a standard (250 µg/ml) (Lowry *et al*. 1951).

Estimation of total carbohydrates

Total carbohydrate content of the samples was estimated by using the method described by Dubois *et al*. (1956).

RESULTS

Biochemical analysis of these three non-conventional oil seed species is shown in **Table 1** and the estimation are expressed in grams per hundred grams of seeds (%). The moisture and ash content of these halophytic oil seed species ranges from 5.59-8.17% and 0.17-1.21%, respectively.

Table 1 Biochemical estimation for the three non-conventional oil seeds species.

Content (g%)	*Cressa cretica*	*Suaeda fruticosa*	*Haloxylon stocksii*
Moisture content	5.87	8.17	5.59
Ash contents	0.72	1.21	0.17
Total protein	33.00	19.09	31.63
Total lipids	3.64	11.83	3.27
Triglyceride	1.90	0.75	1.30
Total carbohydrate	14.00	5.54	14.98

Table 2 Comparison of total lipid content between various oil seed species.

Oil seeds species	Total lipids content (g%)
Cressa cretica	3.64
Haloxylon stocksii	3.27
Suaeda fruticosa	11.83
Glycine max (soybean)	21.00
Z. mays (corn)	4.50
B. napus (canola)	42.70
G. hirsutum (cotton seed)	22.90
Helianthus annus (sunflower)	40.0

In the present study the total lipid contents in *H. stocksii*, *C. cretica* and *S. fruticosa* is in the range from 3.27-11.83% which is lower when compared with the conventional oil seeds (**Table 2**).

The total lipid extract of all the three seed species was fractionated into neutral lipids, phospholipids and glycolipids using chloroform, methanol and acetone, respectively. **Fig. 1A, 1B** and **1C** represents the numbers of peaks corresponding to different components in chloroform, acetone and methanol, respectively for *H. stocksii*. In chloroform fraction four peaks are observed at 455, 460, 465 and 480 nm presenting different neutral lipid classes. In acetone fraction four peaks are detected at 420, 455, 470 and 660 nm representing various glycolipid classes. In contrast, in the methanol fraction only three peaks at 400, 540 and 665 presenting different phospholipid classes were found. **Fig. 2A, 2B** and **2C** represents the number of peaks corresponding to different chloroform, acetone and methanol components, respectively for *C. cretica*. In the chloroform fraction three peaks are observed at 455, 460 and 480 nm representing different neutral lipid classes. In the acetone fraction four peaks are detected at 420, 450, 479 and 660 nm representing various glycolipid classes. In the methanol fraction only two peaks at 420 and 580 nm, representing different phospholipid classes, are observed. **Fig. 3A, 3B** and **3C** represents the numbers of peaks corresponding to different component in chloroform, acetone and methanol, respectively for *S. fruticosa*. In the chloroform fraction three peaks are observed at 455, 460 and 480 nm representing different neutral lipid classes. In the acetone fraction five peaks are detected at 410, 420, 532, 506 and 660 nm representing various glycolipid classes whereas in the methanol fraction only three peaks at 410, 510 and 655 nm, representing different phospholipid classes, are observed.

Among the neutral lipids, triacylglycerol was identified to be the major lipid class component in all three non-conventional oil seed species as observed when thin layer chromatography was performed (**Fig. 4**). Quantitative analysis showed that triacylglycerol in these halophytic oil seed species ranged from 0.75-1.9% (**Table 1**). **Fig. 4** shows all the major classes of neutral lipids were present, including monoacylglycerol, diacylglycerol, sterol, free fatty acid, triacylglycerol and steryl ester.

After extraction of oil from the seeds, the remaining seed meal was analyzed for the total protein content. The protein concentration of *H. stocksii* is higher than that of *C. cretica* and *S. fruticosa* (**Table 3**), ranging from 19.09-33.0%.

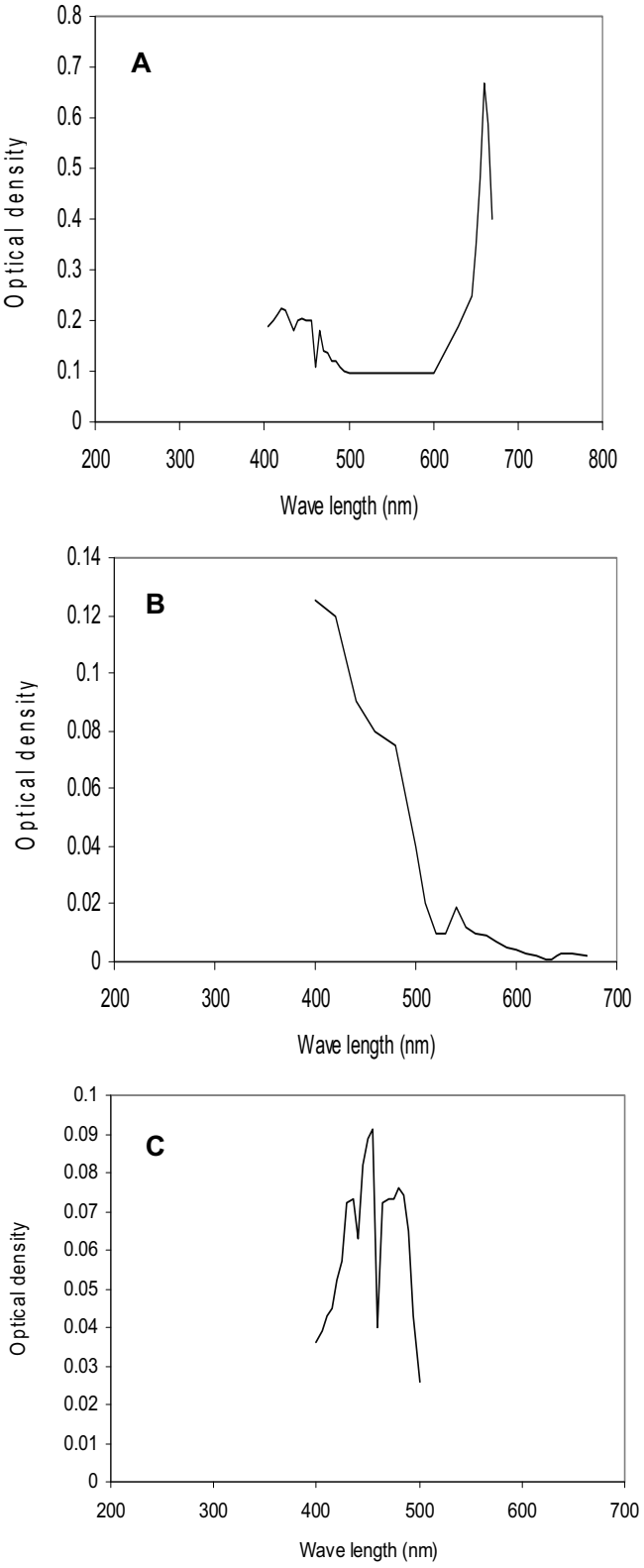

Fig. 1 Fractions of *Haloxylon stocksii*. (**A**) Acetone, (**B**) methanol, (**C**) chloroform fractions.

DISCUSSION

Some halophytic seeds are now being considered as a rich source of oil and are being used as a new potential source of cash crops.

The three terrestrial halophytic seeds acquired locally were evaluated for their biochemical characteristics as oil seed species. In all the parameters there is a great deal of variation among *C. cretica*, *H. stocksii* and *S. fruticosa*. Oil seeds are normally dried to a 7-13% moisture range for good storage and processing (Gunstone and Norris 1983) because in the presence of high moisture content lipases in

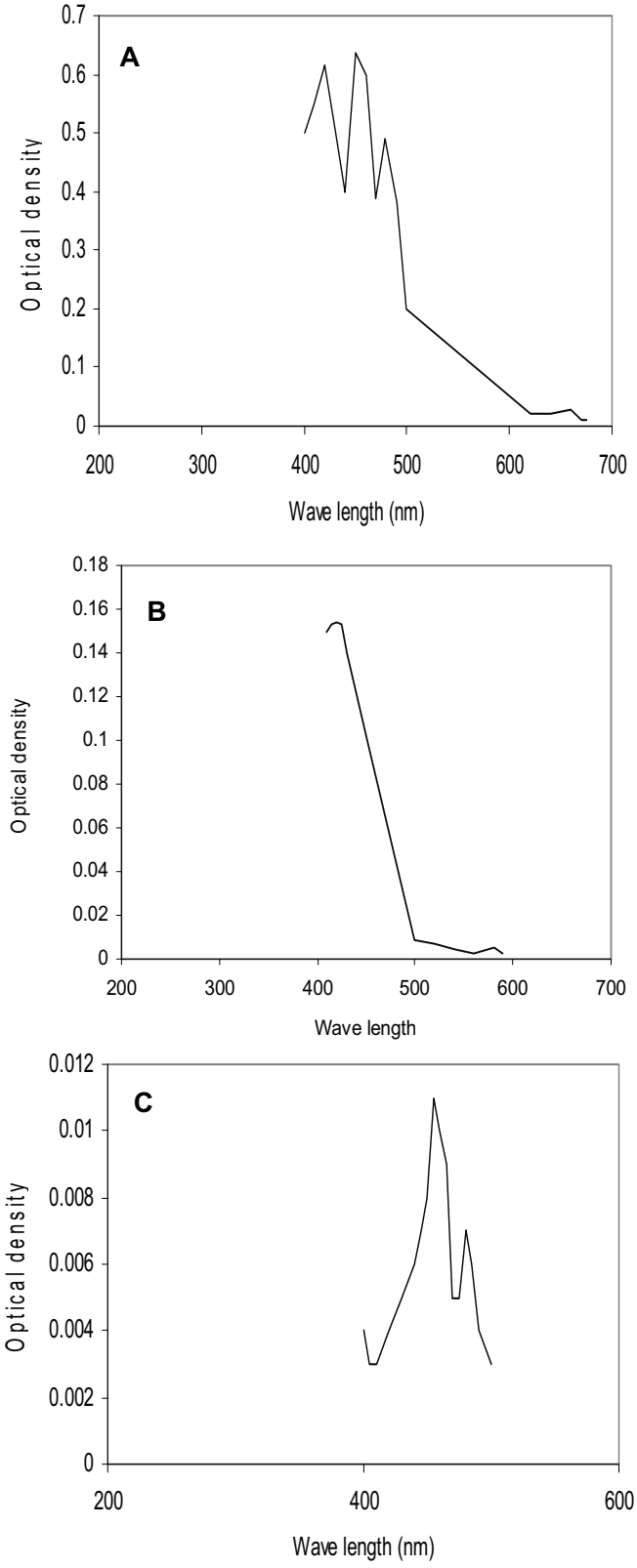

Fig. 2 Fraction of *Cressa cretica*. (**A**) Acetone, (**B**) methanol, (**C**) chloroform fractions.

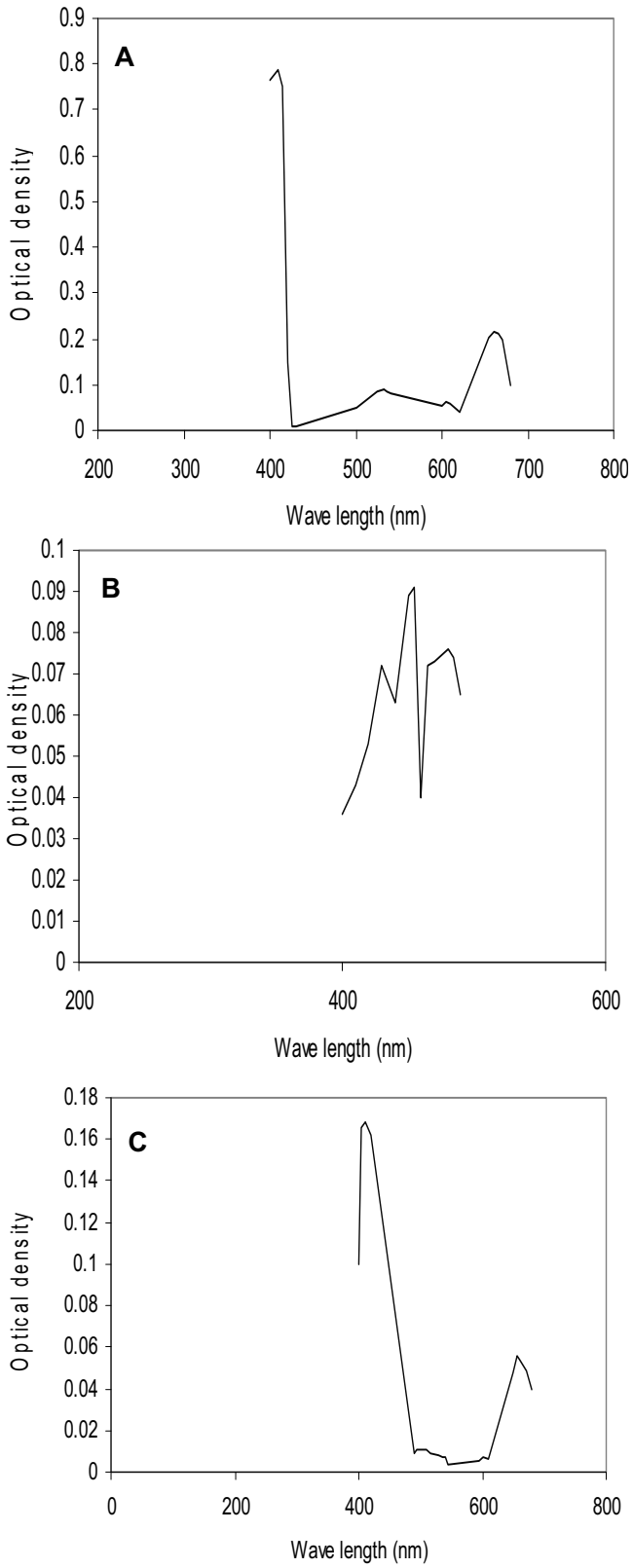

Fig. 3 Fraction of *Suaeda fruticosa*. (**A**) Acetone, (**B**) methanol, (**C**) chloroform fractions.

the seeds breakdown glycerides liberating free fatty acids. Since only intact triglycerides are normally desired for food, any breakdown is unwanted and expensive. The moisture level of all the three halophytic seed species is lower than 7-13% range so these species are good candidates for long-term storage and processing point of view. The ash content is also in very low quantity as compared to the ash content reported for *Salicornia bigelovii* having 5-7% ash content (Glenn *et al*. 1991).

Among many extraction methods available total lipid extraction from these halophytic oil seed species were performed using the modified Bligh and Dyer extraction method (Stobart and Stymne 1990). Using this method very little breakdown of the complex lipids have been observed during the rapid extraction of the seed tissue preparation in modified Bligh and Dyer extraction media. However, among them *S. fruticosa* has a markedly high total lipid content than *Z. mays*. Total oil content reported for *S. bigelovii* is in the range from 26-33% (Glenn *et al*. 1991). *Kosteletzkya virginica* (L.) is another perennial halophytic

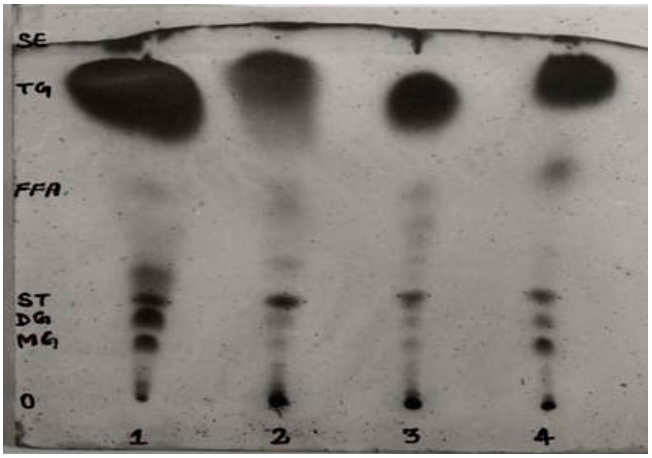

Fig. 4 Thin layer chromatography of neutral lipids of *Z. mays*, *C. cretica*, *H. stocksii* and *S. fruticosa*. **1**: *Z. mays*; **2**: *C. cretica*; **3**: *H. stocksii*; **4**: *S. fruticosa*; **O**: origin; **MG**: monoacylglycerol; **DG**: diacylglycerol; **S**: sterol; **FFA**: free fatty acid; **TG**: triacyglycerol; **SE**: steryl ester.

Table 3 Comparison of total protein content between various oil seed species.

Oil seeds species	Total protein content (g%)
Cressa cretica	33.00
Haloxylon stocksii	31.63
Suaeda fruticosa	19.09
Glycine max (soybean)	40.00
B. napus (canola)	21.40

species, mostly found in tidal marshes of mid Atlantic and southern United States, showed a total percentage content of oil up to 11.28% (He *et al*. 2003). This halophyte was then introduced in China's east coast and the seeds were collected over a ten year period time. It was then reported that the oil content in the unselected mixed and bred lines was 17.53 and 20.64%, respectively (Ruan *et al*. 2008).

Fractionation of neutral lipids of all the three species showed absorbance at three similar wavelengths (455, 460 and 480 nm) confirming similar neutral lipid components but in different quantities. Fractionation of glycolipids of all the three species showed similar absorbance at 420 and 660 nm wavelengths confirming similar glycolipids but in different quantities. Fractionation of phospholipids in all the three species showed different absorbencies at different wavelengths. In the present study all the three non-conventional oil seed species contained variable quantity of neutral lipids, phospholipids and glycolipids. Neutral lipids function as an energy source in various plants and animal tissues whereas; glycolipids and phospholipids are important constituents of cell membranes (Goodwin and Mercer 1985). There are many types of phospholipids which are important for good health and are crucial with their ability to bond to other enzymes as well.

The protein concentration of these three species that ranges from 19.09-33.0% is higher than the protein content in *B. napus* (canola), where as it is nearly equivalent to the seeds of a halophytic plant *S. bigelovii* having 31% protein content (Glenn *et al*. 1991). Due to high protein concentration, the seed cakes or their biomass of the three halophytic seed species can be utilized as a source of protein supplement meals for farm rations. This crude protein cake can also be used as a potential protein source in ruminant diets. The percentage content of crude protein from *Kosteletzkya virginica* is reported to be 8.17%, which is very low as compared to these oil species (He *et al.* 2003).

These halophytic oil bearing seed plant species can be grown using saline resources and can be used as an acceptable substitution for the world's food supply. The residual meal from all these three species can be used as forage or fodder, as well as these three species also offer a suitable feeding resource for oil and protein supplement for wild life. In conclusion, the results described here indicates that these oil seed species could emerge as new potential oil seed crops for coastal and inland saline conditioned areas.

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