Biochemical characteristics, fatty acid profiles and antioxidant activities of tea seed oil

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India, the highest producer of the beverage tea (*Camellia* spp), is not yet self-sufficient on edible oil production. A large portion of oils from secondary sources remains unexploited, which also includes the potentiality of oil production originating from trees. The present study was undertaken to evaluate the possibility of tree-borne oilseeds like tea. The crude fat (oil) was extracted from the dried cotyledons of matured tea seeds of eight different bi-clonal tea seed stocks of Assam, which were commercially maintained for propagation purposes. The oil content ranged from 10.75% to 26.84%. Acid values, iodine values, saponification values and specific gravity of oil were found to be in the range 1.01–1.22 (mg KOH/g), 72.94–94.91 (gI_2/100g), 177.56–200.45 (mg KOH/g) and 0.82–0.88 g/cm^3 respectively. The saturated and unsaturated fatty acids range in tea seed oil as determined by GC-MS was 2.21–20.3% and 79.97–97.79% respectively. The 50% 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (IC_{50} values) of oils of different bi-clonal seed stocks ranged from 60.3 to 81.52 mg/ml. Identification of high level of oleic acid and linoleic acid in most of the tea seed stocks revealed better nutritional quality of tea seed oil. The present study indicates the future scope of tea seed oils as a commercial product in India.

Keywords: Bi-clonal seed, DPPH scavenging activity, saponification, tea, unsaturated fatty acids.

INDIA is one of the largest producers of oilseeds in the world. There has been a persistent gap between demand and domestic availability of edible oils. The Indian Government, with a view to avoid scarcity of this item and consequential rise in prices, has been allowing the import of edible oils. A major reason for the low production of oil seeds is their low productivity when compared to other countries. A large portion of oil from secondary sources remains unexploited, which also includes the potential of oil production from tree origin. At present, production from this source is only 1.2 lakh MT. Vegetable oils from tree-borne oilseeds (TBOs) have potential industrial and domestic usage and can save edible oils from being diverted to these purposes[1].

Even though India is the highest producer of tea, and famous for her characteristic types of tea, no thought has been given for the alternative use of the tea plant. So far, tea seeds have been used for producing planting material. Under normal growing conditions, after planting, seeds can be harvested from the third year onwards. At this stage, the seed yield is a maximum of 6.42 qtls/ha, under triangular planting system with 3 m × 3 m spacing. The yield doubles after 5 years of planting and becomes around six times after 8 years of planting[2].

However, studies involving tea seed oil have already been initiated in other countries. The tea plant produces large oily seeds[3,4]. Tea seed oil can be compared to olive oil (*Olea europae*) in quality and in China, roughly one seventh of the population uses tea seed oil for cooking[5]. The kernels, which make up about 70% of the tea seed weight, are rich in oil[6]. Tea seed oil contains more than 84% unsaturated fatty acids, such as oleic acid, linoleic acid, and linolenic acid, and is characterized by its unique flavour, durable storage, and ease of absorption into the body[7]. It is a source of high level of antioxidants (polyphenols, carotenoids and vitamin E), and emollients for skin care, which minimizes signs of ageing[4,8].

In India, more particularly in Northeast India, all the three basic types of tea, viz. 'Assam', 'China' and 'Cambod' and their hybrids are under cultivation. Thus, there is scope for studying the potentiality of tea seeds for extraction of oil for commercial use, from different types of tea plants. So far, seeds are used only for propagation purposes. This exploratory study has significant importance, as it opens up a new area of study of alternative use of tea plant in India. Studies on quality and quantity of tea seed oil of the commercially cultivated germplasm of Assam may certainly lead to the production of vegetable oils, which may further expose the potential utility of this oil in industrial and domestic usage. Considering the paucity of data of the seed stocks of cultivated germplasm of Assam, India in the above mentioned field, the present study was proposed to evaluate the biochemical and physical properties of tea seed oil.

Tea fruits of different clones were collected from different seed *baris* approved by the Tocklai Tea Research Institute (Jorhat, Assam). The fruits of different clones were collected at two developmental stages, viz. seven and eight months (fully matured) after seed development. The fruits were stored at –20°C until these were used for further analysis.

The chemicals used in the present study were collected from Sisco Research Laboratories Private Limited (Andheri, Mumbai, India). All the chemicals were of analytical grade.

The crude oil content (percentage) was determined according to Association of Official Analytical Chemists (AOAC)[9] using the formula

$$\text{Crude oil content (\%, dry weight basis)} = \frac{(b-a) \times 100}{\text{Weight of the sample (g)}}, \quad (1)$$

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where a is the pre-weighed solvent flask (g) and b the weight of flask with oil after extraction (g). The extracted tea seed oil and the defatted samples were kept in tightly closed vials and stored in a refrigerator at 4°C for further biochemical analysis.

The specific gravity of crude oil was calculated according to AOAC[9] using the formula

$$\text{Specific gravity (g/cm}^3\text{)} = \frac{\text{Mass of 1 ml oil}}{\text{Mass of 1 ml water}} \text{ at 25°C.} \quad (2)$$

The saponification values were calculated according to AOAC[9], using the formula

$$\text{Saponification value (mg KOH/g oil)} = \frac{[(B-S)\times(N)]\times 56.1}{W}, \quad (3)$$

where B is the volume (ml) of 0.5 N HCl required to titrate blank; S volume (ml) of 0.5 N HCl required to titrate the sample; N the normality of HCl solution and W the weight of the sample in grams. The equivalent weight of KOH is 56.1.

The iodine value was determined according to AOAC[9], using the formula

$$\text{Iodine value (g}I_2\text{/100 g oil)} = \frac{A\times N\times 0.1269\times 100}{\text{weight of oil (g)}}, \quad (4)$$

where A is ml of $Na_2S_2O_3$ (sodium thiosulphate) (Blank-Test); N, the normality of $Na_2S_2O_3$ solution; 1 ml of 1.0 N; $Na_2S_2O_3$ = 0.1269 g of iodine. Free fatty acids were determined according to AOAC[9], and expressed using the formula

$$\text{Acid value (mg KOH/g oil)} = \frac{[(A-B)\times N\times 56.1]}{W}, \quad (5)$$

where A is the volume (ml) of standard alkali used in the titration; B the volume (ml) of standard alkali used in titrating the blank; N the normality of standard alkali and W is the weight of the sample (g). The equivalent weight of KOH is 56.1.

Fatty acid methyl esters (FAMEs) of extracted oils were prepared using a fatty acid methylation method[10].

The crude oil, extracted from eight different bi-clonal seed stocks were used to prepare FAMEs. To the culture tube, 150 mg of oil was taken, to which 3 ml of 14% BF_3 was added. The tube was covered with aluminium foil and heated at 83°C for 45 min. The content was then transferred to a 30 ml separating funnel and the washings of the funnel were also collected. It was washed four times with 1 ml portions of hexane, shaken and then allowed to separate. Saturated NaCl solution (4 ml) was then added to the separating funnel. It was then shaken and the hexane layer was collected over Na_2SO_4. The funnel was then rinsed with 2 ml of hexane and the washing was collected along with hexane extract. The hexane extract was then filtered through Whatman No. 4 filter paper. The volume of the extract was then reduced to 2–3 ml using a lyophilizer.

The FAMEs were identified by gas chromatography (GC) using Agilent 7899A GC-FID (Agilent Technologies, Folsom, CA, USA) equipped with 19091 J-413 HP 5–5% phenyl methyl siloxane and a capillary column (30 m × 320 μm × 0.25 μm internal diameter). Mass spectrometer used during the present study was 240 ion trap MS with a mass range of 50–550 m/z.

A solution of 1 μl of methylated sample was injected in splitless mode. The inlet temperature was 220°C and the detector temperature was 260°C. The initial oven temperature was 50°C for 1 min which finally increased to 250°C, at 7°C/min and held at 250°C for 1 min. Helium was used as the carrier gas at a flow rate of 1.4 ml/min. The delay time was 3 min to avoid the peak of the solvent. The FAMEs were identified and quantified by the mass spectra obtained from the mass spectrometer.

The antioxidant activity of tea seed oil extracted was determined[11]. A 2 ml sample of the oil extracted at various concentrations (25–150 mg/ml) in dimethyl sulphoxide (DMSO) was added to 2 ml of 0.005% (w/v) ethanolic 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution. The control was prepared using 2 ml of DMSO in 2 ml of 0.005% DPPH in ethanol, while 2 ml DMSO and 2 ml ethanol served as blank. The decrease in absorbance after incubation for 30 min at 30°C in the dark was measured at 517 nm by a UV/Vis spectrophotometer.

The radical-scavenging ability of the different oil samples was calculated according to the formula

$$\text{DPPH scavenging (\%)} = [(A \text{ control} - A \text{ sample})/A \text{ control}] \times 100,$$

where A control and A sample were defined as absorbance of the control and extracted oil samples respectively. The IC_{50} value is defined as the amount of sample (mg oil/ml DMSO) for which 50% DPPH inhibition is observed. It was calculated from the graph in which the per cent DPPH inhibition was plotted against different concentrations of the samples.

The data obtained from various biochemical analysis were subjected to statistical analysis using 'completely randomized design'[12].

Oil content and quality of oil extracted from matured tea seeds are presented in Table 1. There was a gradual increase in oil content among all the bi-clonal tea seed

Table 1. Oil content (%, dry basis) and quality of oil extracted from matured tea seeds

	Oil percentage		Saponification value (mg KOH/g)		Iodine value (gI_2/100g)		Acid value (mgKOH/g)		Specific gravity (g/cm^3)
Biclonal seed stock	A	B	A	B	A	B	A	B	B
TS-378	18.69	19.96	179.90	179.90	91.49	90.43	1.13	1.13	0.83
TS-379	23.30	26.84	185.64	185.54	84.27	84.20	1.12	1.12	0.86
TS-462	17.13	21.38	192.56	192.36	79.18	78.65	1.01	1.01	0.86
TS-463	13.48	15.86	188.45	188.29	82.41	80.95	1.07	1.07	0.83
TS-464	20.10	20.62	180.43	180.67	81.83	80.79	1.16	1.16	0.86
TS-491	10.75	11.02	200.17	200.45	94.91	93.06	1.22	1.22	0.82
TS-506	18.06	18.96	194.31	194.60	73.54	72.94	1.12	1.12	0.88
TS-520	15.10	15.64	177.78	177.56	87.63	86.55	1.19	1.19	0.82
Mean	17.07	18.75	187.40	187.42	84.40	83.44	1.12	1.12	0.84
S.Ed (±)	2.05	1.69	0.33	0.39	0.40	0.67	0.01	0.01	0.014
CD t 0.05	3.58	2.95	0.59	0.69	0.70	1.18	0.02	0.02	0.025

A, Seven months after seed formation; B. Eight months after seed formation.

Table 2. Composition of fatty acid methyl ester (%) in tea seed oil eight months after seed formation

Tea seed stock	$C_{8:0}$*	$C_{12:0}$*	$C_{14:0}$*	$C_{16:0}$*	$C_{18:0}$*	$C_{18:1}$*	$C_{18:2}$*	$C_{18:3}$*	Total	SFA*	MUFA*	PUFA*	Total
TS-378	–	3.15	0.60	2.48	0.73	49.56	42.98	0.5	100	6.96	49.56	43.48	100
TS-379	–	4.76	0.38	0.2	–	41.93	52.35	0.38	100	5.34	41.93	52.73	100
TS-462	–	–	0.60	2.00	0.60	52.68	41.34	2.78	100	3.2	52.68	44.12	100
TS-463	0.20	5.65	0.69	0.86	–	35.86	55.06	1.68	100	7.4	35.86	56.74	100
TS-464	–	–	–	2.21	–	53.30	44.49	–	100	2.21	53.30	44.49	100
TS-491	–	5.19	–	–	–	30.48	51.44	12.89	100	5.19	30.48	64.33	100
TS-506	–	9.25	–	–	0.26	63.86	25.33	1.30	100	9.51	63.86	26.63	100
TS-520	–	4.53	–	15.50	–	47.94	31.40	0.63	100	20.03	47.94	32.03	100

*$C_{8:0}$, caprylic acid; $C_{12:0}$, lauric acid; $C_{14:0}$, myristic acid; $C_{16:0}$, palmitic acid; $C_{18:0}$, stearic acid; $C_{18:1}$, oleic acid; $C_{18:2}$, linoleic acid; $C_{18:3}$, linolenic acid; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

stocks from seven months to eight months after seed formation. Among the bi-clonal tea seed stocks, TS-379 showed the highest oil content of 23.30% and 26.84% and TS-491 showed the lowest oil content of 10.75% and 11.02% after seven and eight months of seed formation respectively. Based on the seed yield of TS-379 on triangular planting[2], after 8 years of planting, 2857.14 kg seed yield/year/ha can be obtained which is equivalent to 766 litre of oil/ ha.

The oil content of tea seeds of different clones cultivated in Kenya was found to be 17.5–25.2% (ref. 13). Similar oil content (23% and 27.21%) was also reported earlier in tea seeds[14–16].

However, there are reports of higher level of oil (30–32%) in tea seeds[3,17–23]. Saponification values of oil extracted from the bi-clonal seed stocks did not vary during different maturity periods, but there was significant variation among different bi-clonal seed stocks as it ranged between 177.56 and 200.45 mg KOH/g oil. Among the bi-clonal seed stocks, the highest saponification values were found to be 200.17 and 200.45 mg KOH/g in TS-491 and the lowest saponification values were 177.56 and 177.78 mg KOH/g in TS-520 after seven and eight months of seed formation respectively. The difference in saponification values was due to differences in the length of fatty acids present in the oil of these bi-clonal tea seed stocks as presented at Table 2. The saponification value of tea seed oil was also reported to be 181–195 mg KOH/g (refs 3, 18–24).

Iodine values of each bi-clonal seed stock did not vary at different maturity periods, but there was significant variation among different bi-clonal seed stocks. The highest iodine value was 93.06 gI_2/100 g and 94.91 gI_2/100 g in TS-491 and the lowest was 72.94 gI_2/100 g and 73.54 gI_2/100 g in TS-506 after seven and eight months of seed formation respectively. The differences observed in iodine values of tea seed oils of different bi-clonal seed stocks might be due to differences in the composition of fatty acids. This is supported by detection of higher level (64.33%) of polyunsaturated fatty acids (linoleic acid and linolenic acid) in bi-clonal seed stock TS-491 (Table 3) where iodine value was also observed to be the highest.

The present findings on the iodine values of oil extracted from different tea stocks were found to be in agreement with those reported earlier[3,13–16,21]. Since the iodine values were below 100, the oils belonged to the category of non-drying oils.

In the present study, the acid values of the oil extracted from bi-clonal seed stocks did not vary at different maturity periods. The acid value was found to be highest

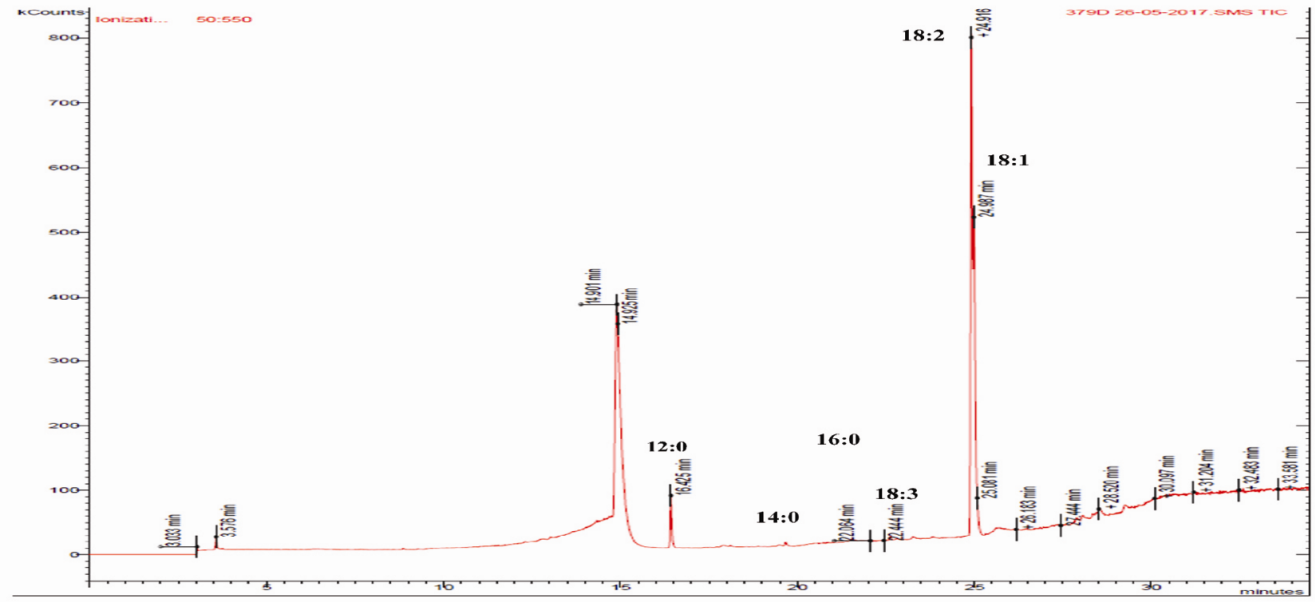

Figure 1. Chromatogram of fatty acid methyl ester of tea seed oil extracted from seeds of TS-379. Peaks are defined as follows: 12 : 0, lauric acid; 14 : 0, myristic acid; 16 : 0, palmitic acid; 18 : 1, oleic acid; 18 : 2, linoleic acid; and 18 : 3, linolenic acid.

in TS-491 (1.22 mg KOH/g) and lowest in TS-462 (1.01 mg KOH/g). Earlier, it was reported that the acid values of tea seed oil ranged from 0.894 to 1.30 mg KOH/g (refs 13, 21).

In the present study, the highest specific gravity was found to be 0.88 g/cm^3 in TS-506 and the lowest was 0.82 g/cm^3 in TS-491 and TS-520. However, the specific gravity in *Camellia oleifera* seed oil at 25°C was reported to be 0.904 g/cm^3 (ref. 14).

The fatty acid profiles of tea seed oil extracted from eight different bi-clonal seed stocks of Assam (Table 2 and Figure 1) indicated the presence of more than 90% unsaturated fatty acids in the total fatty acid methyl ester of the oil. In all the bi-clonal seed stocks, the major fatty acids present were oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$). Earlier, it was reported that tea seed oil contained more than 84% unsaturated fatty acids (UFAs)[24]. However, in other studies, the unsaturated fatty acid content was reported to be 75.89% (ref. 25). The major fatty acids (FA) of tea seed oil was reported to be oleic ($C_{18:1}$), linoleic ($C_{18:2}$), palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids[15,16,26–28].

Mono-unsaturated fatty acids (MUFA), oleic acid was the major fatty acid in five of the eight bi-clonal seed stocks namely TS-462, TS-378, TS-464, TS-506 and TS-520 with a percentage of 52.68%, 49.56%, 53.30%, 63.86% and 47.94% respectively. Earlier it was reported that the major fatty acid in the *Camellia* spp seed oil was oleic acid[3,4,14,21,25,29,30].

In the present study, the highest polyunsaturated fatty acids (PUFA) (64.33%) and the lowest PUFA (26.63%) was found in TS-491 and TS-506 respectively. Earlier, in tea seed oil PUFA were reported to be 24.2%, 22.47% and 27.86% (refs 4, 15, 31).

Linoleic acid was the major fatty acid in three of the eight bi-clonal seed stocks namely TS-379, TS-491 and TS-463 with 52.35%, 51.44% and 55.06% respectively. However, lower amounts (3.5–22.41%) of linoleic acid in tea seed oil were reported earlier[3,14,32,33]. Linolenic acid was also present in all the bi-clonal seed stocks except in TS-464. The highest linolenic acid (12.89%) was found in bi-clonal seed stock TS-491 and the lowest (0.5%) was found in TS-378. Earlier, it was reported that tea seed oil consisted 0.3% linolenic acid[3].

The saturated fatty acids (SFA) ranging between 2.21% and 20.03% were also found to be present in all the bi-clonal seed stocks studied. The saturated fatty acid in tea seed oil was reported to be 20.67% and 21.5% earlier[15,31].

The results of DPPH free radical scavenging assay for tea seed oil extracted from bi-clonal seed stock TS-379 are presented in Figure 2. The concentration of the sample that reduced free radical absorbance by 50% (IC_{50}) served as an index to compare antioxidant activity. The tea seed oil extracted from different bi-clonal seed stocks showed concentration-dependent scavenging of the DPPH free radical. The DPPH percentage inhibition by tea seed oil ranged between 28.57% and 84.62%, 32.60% and 87.13%, 33.47% and 86.69%, 38.93% and 86.47%, 28.68% and 86.91%, 35.87% and 89.09%, 30.20% and 87.45% and 44.16% and 87.02% in TS-462, TS-378, TS-379, TS-491, TS-463, TS-464, TS-506 and TS-520 respectively, when the concentration of extracted oil varied from 25 to 150 mg oil/ml DMSO. The antioxidant activity

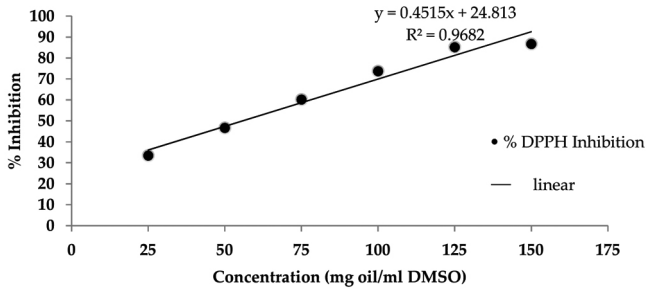

Figure 2. DPPH free radical scavenging activity (%) of tea seed oil extracted from bi-clonal seed stock TS-379.

Table 3. IC_{50} values of tea seed oil from different bi-clonal seed stocks

Bi-clonal seed stock	IC_{50} value (mg/ml)
TS-378	81.52
TS-379	62.30
TS-462	73.52
TS-463	60.30
TS-464	64.89
TS-491	61.30
TS-506	65.13
TS-520	62.18
Mean	66.29
S.Ed (±)	0.16
$CD_{t\ 0.05}$	0.28

of tea (*C. sinensis*) seed oil was reported to be from 12.1 ± 2.7% to 67.8 ± 6.4%, for the oil extracted by Soxhlet, when the concentration of extracted oil varied from 10 to 160 mg/ml respectively[15].

The IC_{50} values of tea seed oil extracted from different bi-clonal seed stocks are presented in Table 3. The IC_{50} values ranged between 60.30 mg/ml (TS-463) and 81.52 mg/ml (TS-378). However, higher IC_{50} values for tea seed oil (146.70, 236.20 and 155.20 mg sample equivalent/ml) were reported[14]. The IC_{50} value of tea seed oil extracted by SC-CO_2 was 35.8 mg/ml, which was nearly 40% lower than that (59.6 mg/ml) of Soxhlet-extracted oil[15]. Earlier, it was suggested that polyphenols were responsible for antioxidant activity, which protected the tea seed oil from damage during storage[4,21,23,25].

Among the tea seed stock studied, TS-379 was found to be the best considering the highest oil content (23.3–26.84%), higher unsaturated fatty acid (>90%) and IC_{50} value for DPPH scavenging being on the lower side. Identification of higher levels of oleic acid and linoleic acid in oil extracted from most of the tea seed stocks, together with antioxidant activity and the IC_{50} values for DPPH scavenging which ranged from 60.3 to 80.52 mg/ml revealed the better nutritive quality of tea seed oil. The findings of the present study may lead to alternative use of tea in the major tea growing regions of India in future.

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ACKNOWLEDGEMENT. K.S. acknowledges the financial help in the form of DST Inspire Fellowship to carry out this study.

Received 7 December 2017; accepted 2 March 2018

doi: 10.18520/cs/v114/i12/2549-2554

Incessant erosion of high tidal mudflats in the northern Gulf of Khambhat

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Extensive erosion of high tidal mudflat along the northern parts of Gulf of Khambhat (GoK) was observed from the analysis of time series satellite images during the time period from March 2014 to September 2017. Around 28.66 sq. km area of high tidal mudflat eroded within this time period. Maximum erosion rates estimated have even peaked to about 4 km/year showing the severity of erosion. The mudflats under erosion are along the outer boundary of a meandering tidal channel connecting the Gulf with Mahi river. A possible cause of the incessant erosion of mudflats is the strong tidal currents along the outer boundary of the meandering tidal channel, that have carved the mudflats and pushed the tidal channel further landward. A subtle seasonal pattern of erosion was observed with decrease in erosion rates during the summer monsoon period when the high tidal currents are weak due to the river influx. Rapid erosion of the tidal mudflats has not only destroyed the vital habitat, but has also eventually exposed the inhabited land area to tidal flooding, making it vulnerable to erosion. The study shows the importance of assessing the stability of mudflats along the GoK, where large development activities are proposed.

Keywords: DSAS, erosion, high tidal mudflat, satellite data, tidal channel.

EROSION of the coastal region poses a major threat not only to the human population, but also to the vital coastal ecosystem. The dynamic interaction between nearshore features and the hydrodynamics of the region, termed as coastal processes, determines the stability of the adjacent shoreline. Moreover, various developments along the coast enhance the changes in the shoreline. Coastal erosion is considered as a major threat worldwide and India, consisting of a long shoreline on the either side of its peninsular is also subjected to erosion in varied strengths[1].

Satellite data has proved its applicability in deciphering various coastal processes by providing synoptic observations with high temporal coverage. Monitoring shoreline changes is essential in understanding the various coastal processes, developmental planning and estimating regional scale sediment erosion and accretion[2,3].

The present study involves monitoring the erosion of high tidal mudflat along the Gulf of Khambhat (GoK) using sequential satellite images. Only limited studies carried out based on satellite observations comprehend the coastal dynamics of GoK[4–6]. GoK is a tidal regime located along the west coast of India, which is a north-south penetration of the Arabian Sea between Saurashtra and the Indian Peninsula (Figure 1). The tide range within the gulf reaches about 10 m at Bhavnagar[7], which is the largest along the Indian coast.

The coastal geomorphology of the gulf is predominantly of tidal mudflats, occupying an area of about 2588 sq. km (refs 8, 9). Although the gulf is a tide dominated region, five major rivers forming an estuary along the coast of Khambhat contribute significantly to its hydrodynamics.

Coastal erosion along the northern part of GoK was monitored using temporal satellite images for a period of 3.5 years. Cloud free Landsat 8 Operational Linear

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