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Determination of β-carotene in jute leaves by spectrophotometry and thin layer chromatography

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Abstract

β-carotene is a carotenoid pigment that converts to vitamin A in human body and plays crucial role in visual functions, reproductive performance and immune system. Jute leaves contain β-carotene but amounts in different varieties of jute leaves are not known. The study was undertaken to estimate the β-carotene content in fresh jute leaves of the cultivated jute species *Corchorus olitorius* L. and *Corchorus capsularis* L. by spectrophotometry and thin layer chromatography. β-carotene content varies in leaves of different varieties of two species. Leaves of some *C. olitorius* variety showed higher amount of β-carotene than the leaves of *C. capsularis* variety. Some varieties of *C. olitorius* showed similarity with some varieties of *C. capsularis*. Variety *Robi*-1 of *C .olitorius* showed the highest content of β-carotene 39.93mg/100g. O-72, O-4, O-3820, O-9897 and OM-1 of olitorius variety showed higher β-carotene than all the varieties of *C. capsularis*. JRO-524 and MG-1 of *C. olitorius* variety is comparable to CVL-1, D-154, BJC-2197 and *deshi pat shak*-1 variety of *C. capsularis* regarding β-carotene content. Lowest β-carotene was estimated 12.55mg/100g in O-795 of olitorius variety and 12.32mg/100g in BJC-83 and 11.34mg/100g in BJC-2142 of capsularis variety. Thin layer chromatography system silica gel on TLC Al foils as stationary phase and hexane as mobile phase performs good separation and identification of β-carotene in jute leaves.

Keywords: β-carotene; Jute leaves; *Corchorus olitorius; Corchorus capsularis*; Spectrophotometry; Thin layer chromatography

1. Introduction

Jute (*Corchorus* sp.) leaves consumed as vegetable in many countries in Asia and Africa [1-3]. Edible species of *Corchorus* are very good source of proteins, vitamins (A, C, E), mineral nutrients like calcium, iron and carotenoid like lycopene [4-6]. Previous research mentioned that jute leaves are rich source of beta carotene [7, 8]. Beta carotenes present as micro-nutrients in fruits and vegetables and are responsible for their bright orange, red and yellow colours. In the plant carotenoids act as photosynthetic accessory pigments and also play a protective function as scavengers of oxygen radicals released from chloroplasts during photosynthesis, thus protecting cellular constituents such as DNA from free radical damage [9, 10]. One of the most physiological functions of carotenoids in human nutrition is to act as pro-vitamin A (vitamin A precursors). Many studies show strong correlations between carotenoids intake and reduced risk of some diseases, such as cancers [11], artherogenesis [12,13], bone calcification [14], eye degeneration [15,16] immune function [17-19] and neuronal damage [20]. Based on the epidemiological studies a positive link is suggested between

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higher dietary intake and tissue concentration of carotenoids and lower risk of chronic diseases [21, 22]. Carotenoids may play a key role as free-radical scavenger and antioxidants in human body tissues [23, 24].

B-carotene belongs to the carotene class, which is one of the most abundant carotenoids found in the diet. Fruits and vegetables constitute major source of carotenoids in human diet [21, 22]. Nutrition rich vegetables get attention in some region like Sub-Saharan Africa to combat malnutrition and famine. Among other vegetables, *Corchorus spp*. has been identified as promising for up-scaling and addressing food security [25]. Therefore, for the perspective of food security, usefulness and nutritional value of indigenous leafy vegetables like jute (*Corchorus spp*.) is required to be rediscovered. There is growing demand of jute leaves for up scaling as its well-known health benefit. Rediscovering nutritional value of jute leaves may be helpful to promote market potentiality as well as awareness among common people. Extraction and estimation procedure of carotenoids are rather complex. High performance liquid chromatography (HPLC) is commonly used for determination of β-carotene. HPLC requires expensive instrument and skillful operation. This work was aimed to investigate the β-carotene content of commercially cultivated jute leaves by simple spectrophotometry and thin layer chromatography (TLC).

2. Material and methods

2.1. Plant sample

Jute leaves of nine varieties of *Corchorus olitorius* and ten varieties of *Corchorus capsularis* were collected from the experimental field of Breeding Division of Bangladesh Jute Research Institute (BJRI), Dhaka, Bangladesh. The site belongs to the Agro Ecological Zone-8 namely, young Brahmaputra and Jamuna Floodplain. Cultural activities were performed according to Chowdhury and Hassan [26]. Fresh healthy jute leaves of selected varieties were harvested from 75 days old jute plant for estimation of β-carotene content. Three biological replications were used for each sample.

2.2. Chemicals and Instruments

- Tetrahydrofuran (THF), Dichloromethane (DCM), NaCl, Na_2CO_3, Hexane and Trans β-carotene (Sigma-Aldrich, Germany).
- Silica gel on TLC Al foils and capillary pipette (Sigma-Aldrich, Germany)
- Centrifuge (Hitachi CR22N high-speed refrigerated centrifuge, Tokyo, Japan) and Spectrophotometer (UV-6300 PC, VWR, Radnor, PA)

2.3. Estimation of β-carotene using Spectrophotometry

Spectrophotometric method for estimation of β-carotene was followed according to Karnjanawipagul et al. [27]. β-carotene from jute leaves samples was extracted by the procedures described by Herrero-Martinez et al. [28] with little modification. Briefly, jute leaves were washed with de-ionized water and chopped. Leaves (10g) were mixed with anhydrous Na_2CO_3 (1g) and homogenized with blender. Homogenized leaves (10g) were transferred into a centrifuge tube and Tetrahydrofuran 20ml was added in it then kept it in the refrigerator overnight. The mixture was centrifuged at 5000rpm for 5min by the centrifuge and supernatant was collected. Dichloromethane 15ml was added to the collected supernatant and then NaCl (10%) (w/v) 15ml was added to it and shaken for two min. The extraction was repeated twice, and organic layer was collected in evaporating bowl and placed in the refrigerator (4°C) for 2/3days for evaporation in cold. The residue was collected and kept at -20°C. Afterwards reconstitute with 5ml DCM and diluted (1/40-fold) with DCM prior measurement by spectrophotometer (UV-6300 PC, VWR, Radnor, PA). β-carotene samples were measured at 461nm.

2.4. Detection of β-carotene using thin layer chromatography

β-carotene was detected by thin layer chromatography using the same sample extracted previously for spectrophotometric measurement. The sample was preserved at -20°C and used for TLC work afterwards. An accurately weighted Trans β-carotene standard was dissolved in DCM to obtain a solution of 100µg/ml. Aliquot of standard solution was applied on TLC plate to compare with jute leaves extract of β-carotene. Silica gel on Al foil was stationary phase and hexane was mobile phase. Capillary pipette was used for placing samples on the TLC plate. Silica gel Al foil sheet were cut as per requirements such as 10cm×8.5cm for sample O1-O5, 10cm×7.5cm for sample O6-O9, 10cm×9cm for sample C1-C5 and 10cm×9cm for sample C6 –C10. A horizontal line was drawn by pencil about 1.2cm above from the bottom of the TLC sheet. Capillary tube was dipped in the sample and gently touched it on the pencil drawn line of the TLC plate to empty it. Several pipettes were used for every individual sample. Several short touches were given to

empty capillary, so the spot was 1-2mm in diameter. Standard Trans β-carotene (100µg/ml) was used on every plate as standard to compare with jute leaf extract β-carotene. Spots were allowed to dry. Afterwards the TLC sheet with spotted end was placed in a beaker in the developing solvent (hexane). The start spotted line was above the solvent level. Then beaker was covered with glass plate. Solvent was allowed to rise up the TLC plate leaving undisturbed until about 80% of TLC plate is wet. The TLC plate was then removed and taken photo immediately.

2.5. Preparation of standard β-carotene solution

Standard Trans β-carotene weighted exactly 0.4mg (400µg) was dissolved in 100ml DCM to obtain 4µg/ml β-carotene solution. This solution was used for calibration curve preparation. The calibration curve was constructed by plotting the concentration versus the corresponding absorbance (Figure 1).

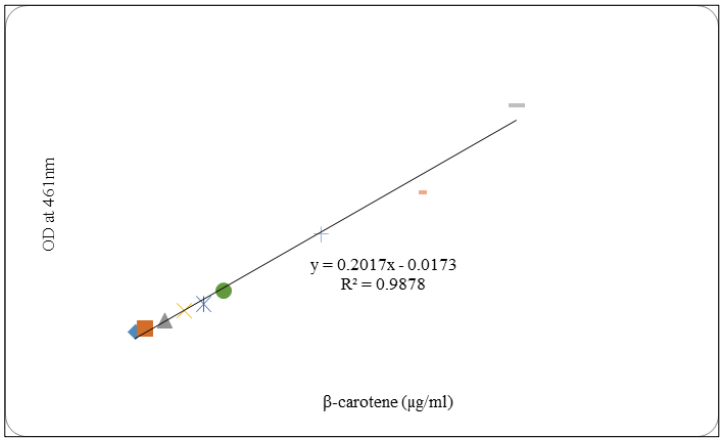

Figure 1 Calibration curves of β-carotene at 0.1 to 4.0µg/ml

3. Results

3.1. Estimation of β-carotene by spectrophotometry

β-carotene content in jute leaves of different varieties of *C. olitorius* and *C. capsularis* were presented in Table 1. Leaves of varieties of *C. olitorius* contain β-carotene 12.55mg/100g to 39.93 mg/100g. Variety*Robi*-1 contain highest amount of β-carotene 39.33mg/100g. Variety O-72, O-4 and O-3820 contain the next highest 30.46, 29.05 and 28.93 mg/100g β-carotene respectively after Robi-1. Variety O-9897, OM-1, JRO-524 and MG-1 have medium amount, 25.67, 24.41, 22.93 and 20.22mg/100g β-carotene and O-795 contain lowest amount of β-carotene 12.55mg/100g in comparison to other *C. olitorius* varieties. β-carotene ranged 11.34 to 22.28mg/100g in leaves of different varieties of *C. capsularis*. Among the *C. capsularis* varieties CVL-1 showed the highest amount of β-carotene 22.28mg/100g. Variety D-154 (21.79mg/100g), BJC-2197 (21.21mg/100g) and *Deshi pat shak*-1 (20.66mg/100g) is comparable to variety CVL-1 having almost same level of β-carotene. Variety BJC-5003 and BJC-7370 contain 19.18mg/100g and 18.08mg/100g β-carotene. BJC-83 and BJC-2142 have lowest β-carotene 11.34mg/100g and 12.32mg/100g respectively.

Table 1 Beta carotene content of leaves ofdifferent *Corchorus olitorius* and *Corchorus capsularis* varieties

Species	Variety	Beta carotene conc. mg/100gm leaves
Corchorus olitorius	O-3820	28.93±0.54
	O-795	12.55±0.46
	O-4	29.05±0.42
	O-72	30.46±0.61
	O-9897	25.67±0.57
	OM-1	24.41±0.38
	MG-1	20.22±0.61

	JRO-524	22.93±0.60
	Robi-1	39.93±0.49
Corchorus capsularis	CVL-1	22.28±0.59
	CVE-3	15.87±0.42
	D-154	21.79±0.42
	CC-45	17.54±0.60
	BJC-2142	11.34±0.34
	BJC-83	12.32±0.44
	BJC-7370	18.08±0.51
	BJC-5003	19.18±0.49
	BJC-2197	21.21±0.35
	Deshi pat shak-1	20.66±0.50

3.2. Detection of β-carotene in jute leaves by TLC

Figure 2 represents the thin layer chromatographic results of β-carotene in different varieties of *C. olitorius* leaves. Figure 3 represent the β-carotene in different varieties of *C. capsularis* leaves. It is observed in every TLC plate that β-carotene of the leaf sample reached to the similar position as the standard β-carotene did and orange colour of all the samples were distinct. Rf value of the jute leaves β-carotene is 0.82 in Table-2 (TLC plate-1) and 0.85 in Tbale-2 (TLC plate-2, 3and 4). Rx value of jute leaves β-carotene was 1.

Table 2 Rf and Rx of Beta carotene of different varieties of jute leaves in TLC plate

Jute species	TLC plate (Figure)	Sample designation	Rf	Rx
C. olitorius	Plate 1 (Fig. 2a)	Bc (standard β-carotene)	0.82	
		O_1 (O-795)	0.82	1
		O_2 (O-9897)	0.82	1
		O_3 (O-72)	0.82	1
		O_4 (O-4)	0.82	1
		O_5 (OM-1)	0.82	1
	Plate 2 (Fig. 2b)	Bc (standard β-carotene)	0.85	
		O_6 (O-3820)	0.85	1
		O_7 (MG-1)	0.85	1
		O_8 (Robi-1)	0.85	1
		O_9 (JRO-524)	0.85	1
C. capsularis	Plate 3 (Fig. 3a)	Bc (standard β-carotene)	0.85	
		C_1 (CVL-1)	0.85	1
		C_2 (D-154)	0.85	1
		C_3 (BJC-7370)	0.85	1
		C_4 (CC-45)	0.85	1
		C_5 (BJC-2197)	0.85	1

	Plate -4 (Fig. 3b)	Bc (standard β-carotene)	0.85	
		C_6 (Deshi pat shak-1)	0.85	1
		C_7 (BJC-83)	0.85	1
		C_8 (BJC-2142)	0.85	1
		C_9 (BJC-5003)	0.85	1
		C_{10} (CVE-3)	0.85	1

4. Discussion

B-carotene content shows variation in different varieties of jute leaves. Most of olitorius leaves contain higher β-carotene than the β-carotene content in leaves of *C. capsularis*. In some varieties of *C. olitorius* shows similarity with some *C. capsularis* varieties. MG-1 (20.22mg/100g) and JRO-524 (22.93) of *C. olitorius* varieties shows β-carotene content like varieties CVL-1(22.28mg/100g), D-154 (21.79mg/100g), BJC-2197 (21.2mg/100g) and *deshi pat shak*-1 (20.66mg/100g) of capsularis varieties. Olitorius variety O-795 shows β-carotene content (12.55mg/100g) like capsularis varieties BJC-2142 (11.34mg/100g) and BJC-83 (12.32mg/100g). Choudhary and his colleagues [29] mentioned that *C. olitorius* showed the highest amount of β-carotene among the 17 genotypes belonging to six jute species. They investigated β-carotene content in different Corchorus species including some varieties of *C. olitorius*. They observed most of the *C. olitorius* varieties have higher amount of β-carotene, some were similar, and some were lower than a capsularis variety (Table-3). In this study, jute leaves of different varieties showed higher amount of β-carotene (Table 1) than the previous report presented in Table-3 [29-31]. This may be occurred due to the geographical origin, genetic make-up, climate, soil condition and structure and fertilizers [32].

Table 3 Beta carotene content in jute leaves in previous report

Species	Condition	β-carotene content	Reference
C. olitorius	Fresh leaves	9774.90µg/100g	Traoré *et al*., 2019 [30]
	Cooked using ash leachet for 15 and 30min	3604.00µg/100g and 3768.80µg/100g	
	Cooked in water for 15 and 30min	2285.50µg/100g and 2365.00µg/100g	
	Fried for 5min following 30min cooking	86.42%	
C. fascicularis	Fresh leaves	62.67 mg/kg	Choudhary *et al*., 2013 [29]
C. triloculairs		36.00	
C. aestuans		76.33	
C. tridens		47.00	
C. capsularis (Maniksari)		61.33	
C. olitorius (Sudan Green)		81.33	
C. olitorius (Tanganyika)		79.70	
C. olitoruius (TJ-40)		64.00	
C. olitorius (Bidhan Rupali)		34.33	
C. olitorius (KOM -62)		63.67	
C. olitorius (JRO-66)		67.33	
C. olitorius (JRO-3690)		66.00	

C. olitorius (JRO-8432)		72.67	
C. olitorius (JRO-204)		51.00	
C. olitorius (JRO-524)		74.00	
C. olitorius (JRO-632)		53.33	
C. olitorius (S-19)		50.67	
C. capsularis (CVL-1)	Fresh leaves	7.82±0.16 mg/100g	Ali et al., 2020 [31]
	Shade dried	4.65±0.32	
	Solar dried	4.42±0.28	
	Cooked	3.81±0.42	

Thin layer chromatography results show the presence of β-carotene content in jute leaves. B-carotene from the leaf samples reached to the position which similar to the position of standard trans-beta carotene sample. Orange colour of the spot was visualized immediately in each variety of leaf samples in every TLC plate (Fig. 2 and 3). β-carotene is bright yellowish-orange due to a system of conjugated double bonds in its structure, it could be identified visually on the chromatogram without using any detecting reagents. The same occurrence observed during this experiment. The spots colours were visualized without any detecting reagent. Rf value of experimented samples were calculated high (0.82/85). It was mentioned in previous report that non oxygenated carotenoids like β-carotene have higher Rf value [33, 34]. Rx value is calculated 1.0 for all the β-carotene in leaves of different varieties of *C. olitorius* and *C. capsularis* (Table-2). Trans β-carotene was used as standard, so the Rx results show clear indication of presence of Trans β-carotene in fresh jute leaves.

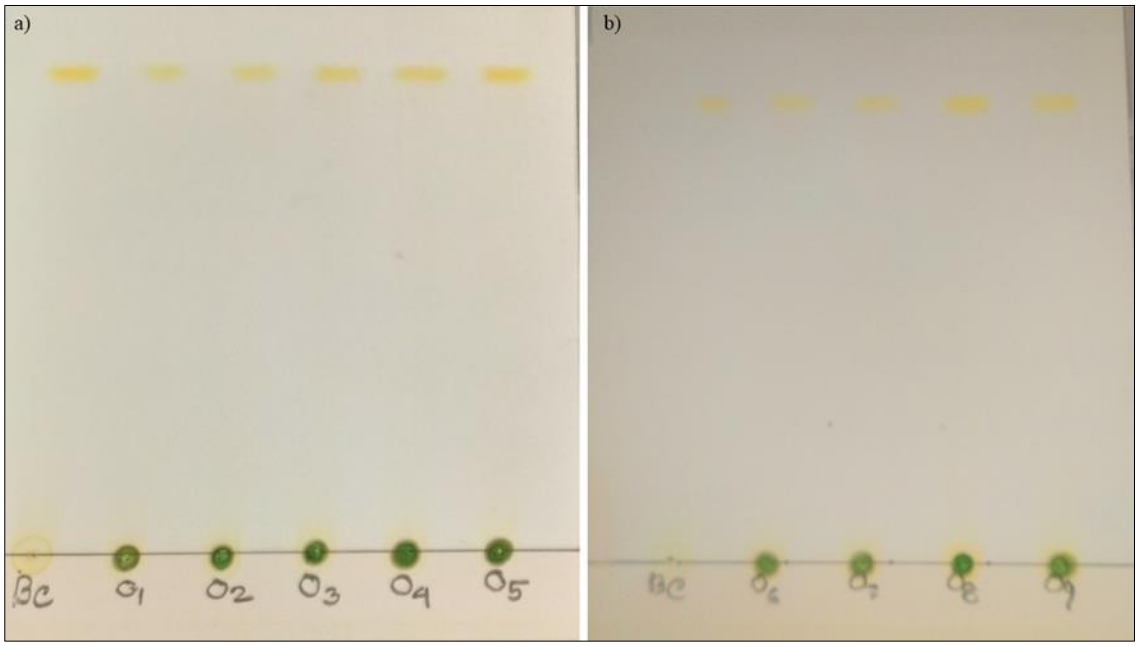

Figure 2 β-carotene detection of nine varieties of *C. olitorius* leaves extract by TLC a) Bc, standard β-carotene; O_1, O-795; O_2, O-9897; O_3, O-72; O_4, O-4; O_5, OM-1 b) Bc, standard β-carotene; O_6, O-3820; O_7, MG-1; O_8, Robi-1; O_9, JRO-524

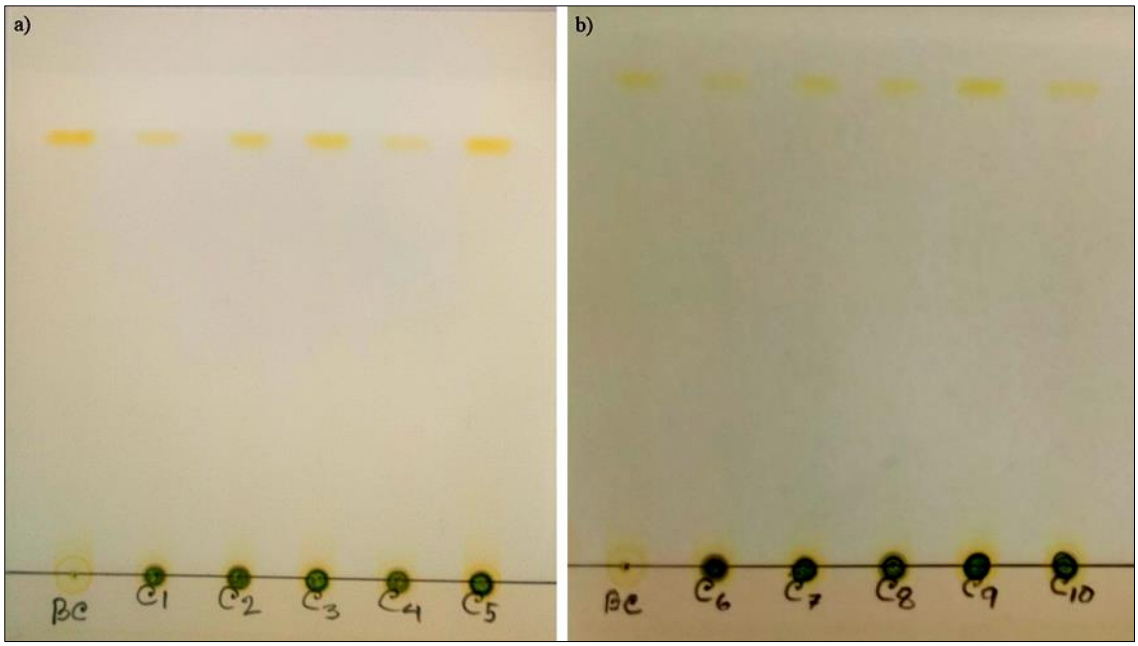

Figure 3 β-carotene detection of ten varieties of *C. capsularis* leaves extract by TLC a) Bc, standard β-carotene; C_1, CVL-1; C_2, D-154; C_3, BJC-7370 C_4, CC-45; C_5, BJC-2197 b) Bc, standard β-carotene; C_6, *Deshi pat shak*-1; C_7, BJC-83; C_8, BJC-2142; C_9, BJC-5003; C_{10}, CVE-3

Carotenoids are insoluble in water but soluble in organic solvents. They present specific absorption spectra that help for their identification. In contact with air, they self-oxidate quickly and degrade [35]. Carotenoids require specific extraction and separation procedures due to its wide range of polarity. β-carotene is practically insoluble in water and hardly soluble even in methanol, acetonitrile or dimethyl sulfoxide [36-38]. Solubility is better in hexane, chloroform and dichloromethane (DCM), highest solubility was observed in Tetrahydrofuran (THF). In this experiment THF was used following DCM twice. So, the extraction process may be performed perfectly that may contribute for obtaining better results of β-carotene by spectrophotometer as well as by TLC. Extraction of carotenoids must be performed very quickly, avoiding exposure to light, oxygen and high temperature [39, 40]. Nitrogen atmosphere is suggested for extraction of β-carotene to maintain cold environment. Here in this experiment cold environment was maintained in refrigerator. Extracted sample was dried at 4°C in refrigerator for 2/3 days. The procedure followed neither nitrogen atmosphere nor quick procedure as mentioned in protocol, but it worked well. Thin layer chromatography results showed that good extraction was performed. TLC method is applied recently for separation and identification of complicated compounds such as carotenoids [41, 42]. In Thin layer chromatography stationary phases are polar and mobile phases are nonpolar or low polarity. Early TLC methods used silica as stationary phase and a nonpolar mobile phase for separation of carotenoids [43-46]. It was also suggested that best separations could be achieved by use of adsorption TLC on alumina with hexane-chloroform mixtures as mobile phases [47]. In most cases petroleum ether with acetone was used major organic mobile phase [48-49]. Hexane was found to be the second major mobile phase used in TLC of carotenoids from plant sources [50, 51]. In this TLC experiment silica gel on TLC Al foils were used as stationary phase and hexane was the non-polar mobile phase. B-carotene in jute leaves were detected well by this TLC system. Thin layer chromatography showed potentiality for separation and identification of carotenoids in vegetable and biological samples [52]. Our TLC reports for β-carotene detection of jute leaves are in agreement with this report.

5. Conclusion

Jute leaves are very rich in β-carotene content. Variation was found in β-carotene in jute leaves of different varieties. Most of the varieties in *Corchorus olitorius* species showed higher β-carotene than the varieties of *Corchorus capsularis*. In TLC system silica gel on TLC Al foils as stationary phase and hexane as mobile phase performed good separation and identification of β-carotene in jute leaves.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that they have no conflict of interest.

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