



## Studies on selected cotton seed (*Gossypium* sp) varieties nutrient profile for human consumption in Tamil Nadu

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**ABSTRACT:** Cotton is one of the important cash crops in India. Cotton seed and its byproducts are utilized for animal feed due to good sources of protein, fatty acid components, vitamins, in addition to the toxic components of gossypol present in it. In the present investigation for the proximate composition, antioxidants, fatty acid profile and gossypol content were analyzed from eight cotton seed varieties at Tamil Nadu to screen for better nutrition and low gossypol content to human consumption. Among the cottonseed varieties, SVPR 2 had a highly significant amount of crude protein and ash, whereas the variety MCU 5 had a high fat content and energy value. These varieties also possess high levels of total phenols, antioxidant activity and  $\alpha$ -tocopherol content. Fatty acid profile analysis revealed that the selected samples contained 24.1 to 33.49 per cent of saturated fatty acids and 64.65 to 69.31 per cent of unsaturated fatty acids. The gossypol content ranged from 14.93 to 88.46mg/100g and for the samples SVPR 2, SVPR 3, SVPR 4, K 2, K 11 and MCU 5 and the gossypol content was within the permissible intake limitation as set by the United State Food and Drug Administration (USFDA - 450mg/kg) and United Nations Food and Agriculture/World Health Organizations (FAO/WHO - 600mg/kg). Taking into consideration the nutrient composition and gossypol content, the cottonseed varieties of SVPR 2 and MCU 5 and its derived products can be promoted as suitable for animal and human consumption to meet better nutrition.

**Key words:** Cottonseed, fatty acid profile, gossypol, nutrient content

Cotton (*Gossypium* sp) is an important cash crop for utilization of natural fiber in textile industries and major cultivated crop in India from ancient period. Among the cotton varieties, *Gossypium hirsutum* (*G. hirsutum*) is the most economical and important species which occupies more than 90 per cent of the world's cotton cultivation (Bellaloui, 2013). In worldwide, production of cotton was 120.86 million bales and largest cotton producing countries was India at 6205 thousand metric tons (TMT), China 5987 TMT, United States of America 4555 TMT and Brazil 1894 TMT in the year of 2017-2018 (Statista, 2019a) and it accounted for 78.87 per cent of global output both in the form of fiber and food supplement to animal feed. After obtaining fiber from the cotton plant, cottonseed is a major product and important source for the production of cottonseed oil and globally its production during 2019 was reported to be 5.16 million

metric tons volume (Statista, 2019b).

Nearly 821 million people are under nourished due to those who are exposed to inadequate food supply along with protein malnutrition (WHO, 2018). Cottonseed is one of the potential food for meeting the nutrition requirement of the world for improving nutritional status but has not been exploited as a source of human nutrition (Agarwal *et al.*, 2003). Cottonseed is a good source of energy, crude protein, fiber content and fat (Piccinelli *et al.*, 2007; Bolek *et al.*, 2016) and its byproducts have a high biological value (55-68%) and safe fatty acid profile such as 50 per cent monounsaturated fatty acid, 21 per cent polyunsaturated fatty acid and 29 per cent saturated fatty acid which conforms to human health guidelines (Bertrand *et al.*, 2005; Prasad and Blaise, 2020). Among the various edible oils, cottonseed oil occupies the second position in  $\alpha$ -tocopherol content than sunflower oil. Value added

products from cottonseed are regularly used as animal feed and the oil is used for human consumption. The cottonseeds as a food in the form of oil and milk for human consumption in southern region of India. Under developed countries were utilizing the cottonseed in directly or indirectly as a source of protein to address the problem of Kwashiorker. However, cottonseeds contain toxic compounds such as gossypol with concentration ranging from 0.02 to 6.64 per cent among the various cotton varieties (Gadelha *et al.*, 2014; Gandhi, 2015; Knutsen *et al.*, 2017). The maximum permissible consumption level of gossypol as regulated by the United State Food and Drug Administration (USFDA) is 450mg/kg by the Food and Agricultural Organization/World Health Organization (FAO/WHO) as 600mg/kg in cotton seed-based products to humans (Prasad and Blaise, 2020).

Although the high biological value of the protein and beneficial fatty acid in cottonseed and its byproducts are encouraging, its utilization as food is minimal due to its gossypol content depends on the varieties. Hence, the main objective of this study is to evaluate the nutrients and gossypol content of the selected cottonseed varieties for better utilization of specific cottonseed variety and its byproducts for animal as well as human nutrition.

## MATERIALS AND METHODS

From the different regions of Tamil Nadu, India the following eight majorly cultivated matured cottonseed varieties were collected from Department of Cotton, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The selected cotton seed varieties included SVPR 2, SVPR 3, SVPR 4, SVPR 5, K 2, K 9, K 11 and MCU 5, which were stored under 4°C for further use. At the time of analysis, the samples were ground and passed through 1 mm BS mesh sieve. All chemicals were procured from Merck, India. The fatty acid methyl esters (FAME) mix, HPLC grade solvents and USP grade standards (gossypol and  $\alpha$ -tocopherol) were procured from Sigma Aldrich, USA.

**Proximate analysis :** The moisture content was estimated after drying of cottonseed at 105°C upto the concordant values (AOAC, 2000). Crude nitrogen and crude protein (Nitrogen x 6.25) were estimated as per the Kjeldahl method (AOAC, 2000). Crude fat was estimated with hexane by Soxhlet apparatus and ash content (gravimetric) were estimated based on the methods of (AOAC, 2000). Crude carbohydrate was estimated by the anthrone method (Hedge and Hofreiter, 1962) and Gross energy was calculated by as per the below formula (Eknayake *et al.*, 1999).

$$\begin{aligned} \text{Gross energy (kJ/100g)} &= (\text{crude protein} \times 16.7) \\ &+ (\text{crude lipid} \times 37.7) + \\ &(\text{crude carbohydrate} \times 16.7) \end{aligned}$$

**Phytochemicals :** Total phenolic content was estimated by the Folin - Ciocalteu reagent method (Singleton *et al.*, 1999) and it was expressed as Gallic Acid Equivalent (GAE) g/100g. The antioxidant activity was estimated by the 2, 2, diphenyl-1-picrylhydrazyl (DPPH) assay (Lim *et al.*, 2007) and the result was expressed as a percentage of Radical Scavenging Activity (% RSA) by the methanolic extract of the samples.  $\alpha$ -tocopherols can be estimated by spectrophotometric method (Kayden *et al.*, 1973) by the following formula.

Amount of $\alpha$ -tocopherol in $\mu\text{g/g}$ of sample	Reading of test at 520 nm –	X
	Reading of test at 420 nm	0.29 X
	Reading of standard at 520 nm	0.15 X
	Total volume of homogenate	
	Volume used X Weight of the Sample	

**Fatty acid profile :** Fatty acid profile was analyzed by (AOAC, 2000) and as Gas Chromatography-Mass Spectroscopy (GC-MS). Lipids were extracted from the sample by a Soxhlet apparatus for 4 hours using hexane. A solution of oil (0.1g) in hexane (10ml) was mixed with 0.2ml of 2N methanolic potassium hydroxide by shaking vigorously for 30 seconds vortex and allows it for overnight. The upper part

of the hexane layer was directly injected into the GC-MS system. The fatty acid methyl esters (FAME) mix (C4 - C24) was used as standard. The fatty acid profiles were analyzed by Rxi-5 Sil MS fused silica column in GC-MS. The analysis condition was: The injection, transfer line, and ion source temperatures were 250°C, 240°C and 230°C, respectively and ultra-high purity helium (99.99%) was used as carrier gas at a constant flow rate of 1.0 ml/ min. 1.0 µL of the sample was injected into the system in split mode (30:1). The quantification of fatty acids was done with respect to the retention time and spectral matching of corresponding FAME standard. The obtained chromatograms were analyzed with the Shimadzu lab solutions software.

**Gossypol content :** The gossypol content was estimated by ultra-high pressure liquid chromatography (UHPLC) from the methanolic extracts of the sample (Gandhi, 2015). About 0.1 g of powdered samples were macerated with acetone upto 16 hours and then filtered in Whatman number 3 filter paper. The extract was evaporated at 50°C. The residue was mixed in 3ml of 100 per cent methanol. The extracts were filtered through a 0.45µm Millipore filter before injection into the column. The UHPLC system consists of the binary pump, degassing unit, thermostatic autosampler and column oven used to separate the analytes. The analytical column was C18 and it was thermostatically controlled at 25°C, the flow rate at 1.0ml/min and the sample and standard injection volume was 2µl. The mobile phase solvents consisted of methanol/Trifluoroacetic acid - 99.8/0.2 (v/v). Detection and quantification were performed at 450 nm in the UV-VIS detector. The identification of gossypol content in the samples was done with the retention time of the corresponding external standard of gossypol.

**Statistical analysis :** The statistical analyses were performed by SPSS 17.0 for the

one way analysis of variance (ANOVA). The results are based on the mean value from three analytical values and the standard deviations. The significance of the difference ( $p < 0.05$ ) was evaluated by the influence of the variety of nutrient composition and gossypol content.

## RESULTS AND DISCUSSION

Oilseeds are good sources of carbohydrate, protein, fat, minerals and vitamins, particularly for vegetarian consumers worldwide. The results of the nutrient composition such as moisture, carbohydrate, crude protein, crude fat, ash, energy and phytochemicals such as total phenols,  $\alpha$ -tocopherol and antioxidant activity were presented in Table 1. The moisture content of all the varieties was approximately 7 per cent, which indicates that cottonseeds were procured from uniform agro-climatic conditions and storage behavior. The carbohydrate content ranged between 64.19 per cent in MCU 5 and 73.99 per cent in SVPR 2. The protein content of SVPR 5 was highly significant compared to other SVPR varieties. The MCU 5 and K 11 variety of cottonseed had the highest protein content of 23.18 and 22.53 per cent, respectively, compared to other cottonseed varieties. The same findings in terms of protein content were reported by (Bertrand *et al.*, 2005), wherein the scientists had analyzed three types of genetically modified varieties and traditional varieties of cottonseeds, cultivated during the years of 1999 and 2000. The crude lipid content was varied from 7.34 to 9.27 per cent, among the different varieties of cottonseed; MCU 5 contained 8.65 per cent of lipid content, whereas the ash content was found to vary between 3.86 to 4.05 per cent. The lipid content of the selected cottonseed varieties was similar to the values obtained for the different genotypes of *G. herbaceum* L. seeds (Gandhi, 2015). The selected cottonseed lines (84027 and 84033) and their parental line (DP62) possessed proximate characteristics such as moisture 7-8.5

**Table 1.** Nutrient profile for selected cottonseed varieties

Nutrient composition	Varieties									
	SVPR 2	SVPR 3	SVPR 4	SVPR 5	K 2	K 9	K 11	MCU 5		
Moisture (%)	7.48±0.16ab	7.56±0.01ab	7.19±0.15a	7.44±0.12ab	7.69±0.17b	7.53±0.07ab	7.77±0.03b	7.63±0.07b		
Carbohydrate (g/100g)	72.47±1.37d	73.99±0.11d	73.26±1.25d	67.6±0.38bc	69.62±0.61c	68.25±0.31bc	65.66±1.09ab	64.19±1.36a		
Crude protein (g/100g)	14.29±0.36a	14.70±0.19a	14.25±0.21a	20.94±0.23d	18.57±0.07b	19.91±0.10c	22.53±0.25e	23.18±0.26e		
Crude lipid (g/100g)	9.27±0.04e	7.34±0.17a	8.63±0.13d	7.51±0.16ab	7.76±0.09abc	7.82±0.16bc	7.96±0.19c	8.65±0.04d		
Ash (g/100g)	3.97±0.08ab	3.97±0.01ab	3.86±0.03a	3.95±0.01ab	4.05±0.07b	4.02±0.01b	3.85±0.07a	3.98±0.02ab		
Energy value (kJ/100g)	1798.37±46.14	1757.84±2.74	1786.76±21.38	1761.74±10.08	1765.32±11.02	1767.08±27.58	1772.86±15.68	1772.86±26.75		
Total phenols (GAE g/100g)	2.25±0.01d	1.98±0.01b	2.02±0.01b	1.96±0.06b	2.10±0.03c	1.85±0.01a	1.86±0.06a	2.68±0.09d		
α-tocopherol (mg/100g)	36.89±1.15e	33.83±0.30c	33.31±0.18c	33.37±1.17c	28.47±0.15a	28.15±0.65a	30.04±0.92b	34.61±0.43d		
Antioxidant activity (% of RSA)	67.25±1.97bcd	64.98±1.17bc	70.59±3.05d	63.76±2.12b	75.48±1.97d	68.00±2.38cd	58.05±0.41a	77.45±2.65d		

Each value mentioned as Mean±SD of three replicate analysis (n=3)

The values mentioned in superscripts are significantly differed at p<0.05 in the same row

**Table 2.** Fatty acid profile for selected cottonseed varieties (%)

Fatty acid profile (%)	Varieties							
	SVPR 2	SVPR 3	SVPR 4	SVPR 5	K 2	K 9	K 11	MCU 5
<b>Saturated fatty acid</b>								
Myristic acid (C14:0)	0.73c	0.58b	0.84d	0.86d	0.98e	0.97e	0.48a	0.83d
Palmitic acid (C16:0)	24.96b	20.82a	24.96bc	28.24f	27.26e	26.45e	25.58cd	25.85e
Stearic acid (C18:0)	2.88c	1.50a	2.82c	3.21d	3.34d	3.47e	2.78c	2.68b
Arachidic acid (C20:0)	1.04f	1.04g	0.96d	0.98e	0.76c	0.71b	0.77c	0.23a
Behenic acid (C22:0)	0.17c	0.16b	0.17c	0.20d	0.15a	0.17c	0.15a	0.16b
<b>Mono unsaturated fatty acid</b>								
Palmitoleic acid (C16:1)	0.63c	0.36a	0.57b	0.65c	0.75e	0.69d	1.24f	0.55b
Oleic acid (C18:1) cis-9	0.40a	0.38a	0.36a	0.33a	20.79c	20.61c	26.14d	19.07b
Vaccenic acid (C18:1) cis-11	20.88c	39.75d	18.34b	18.69b	0.32a	0.21a	0.29a	0.18a
<b>Poly unsaturated fatty acid</b>								
Linoleic acid (C18:2)	47.15d	28.82a	48.48e	44.98c	44.09c	44.81c	40.16b	47.55d

Each value mentioned as Mean of three replicate analysis (n=3)

The values mentioned in superscripts are significantly differed at  $p < 0.05$  in the same row

Saturated Fatty Acid (C14:0, C16:0, C18:0, C20:0, C22:0); Monounsaturated Fatty Acid (C16:1, C18:1(cis-9), C18:1(cis-11));

Poly Unsaturated Fatty Acid (C18:2)

per cent, protein 19-24 per cent, fiber 15-19 per cent, fat 20-23 per cent, ash 3-4 per cent, and carbohydrate 43-47 per cent. The same streaks of nutrients were presented in all the selected cottonseed varieties except for carbohydrate and fat content (Bertrand *et al.*, 2005; Bellaloui *et al.*, 2015; Prasad and Blaise, 2020).

The selected cotton seed varieties had total phenolic content which ranged from 1.96 to 2.72 GAE g/100g, with the highest value recorded in MCU 5 (2.68 GAE g/100g) followed by SVPR 2 (2.25 GAE g/100g) and lowest in K 11 variety (1.86 GAE g/100g). Phenolic compounds are essential in plant constituents because of their effectiveness in radical scavenging property and are positively correlated with antioxidant activity (Heim *et al.*, 2002). In general, cottonseed oil contains an abundant quantity of  $\alpha$  and  $\beta$ -tocopherol, which acts as a natural antioxidant for preventing the oxidation of unsaturated fatty acids (UFA) (Basturk *et al.*, 2007; Talpur *et al.*, 2014). Among the eight varieties of the whole cottonseed, the  $\alpha$ -tocopherol concentration was high in SVPR 2 - 36.89mg/100g followed by MCU 5-34.61mg/100g and low in K 9-28.15 mg/100g. These results were coordinated with the upland 18 cultivars and strains of cottonseeds, which contained  $\alpha$ -tocopherol on average of

125 $\mu$ g/g to 224 $\mu$ g/g in fresh weight, which was cultivated in the year 1997 and 1998, respectively (Smith and Creelman, 2001). The methanolic extracts of the selected samples have shown a scavenging activity, which accounted for 58.05 per cent RSA in K 11 and 77.45 percent RSA in MCU 5, whereas the samples K 2 and MCU 5 were highly significant with K 9 exhibiting 58.05 percent RSA. The results were similar to cottonseed protein hydrolysate and its fractions (Gao *et al.*, 2010). These outcomes indicated that a notable quantity of free radical scavenging activity was presented in cottonseed due to the presence of gossypol, tocopherols, and phenolic content.

The fatty acid profile of the selected samples were presented in Table 3. The fatty acid composition as saturated fatty acids (SFA), C14:0, C16:0, C18:0, C20:0 and C22:0 were accounting for 0.58-0.98 percent, 20.82-28.24, 1.50-3.47, 0.23-1.04 and 0.15-0.20 per cent respectively and UFA including of monounsaturated fatty acids (MUFA) as (C16:1-0.36 to 1.24%), (C18:1 (cis-9) - 0.33 to 26.14%) and (cis-11 on 0.18 to 39.75%) and polyunsaturated fatty acid (PUFA) as C18:2 (28.82%-48.48%) and other UFA's such as C22:1 and C18:3 were not noticeable in selected cottonseed varieties. The above findings were



confirmed in designated cottonseed genotypes (Bertrand *et al.*, 2005; Roy *et al.*, 2012). Gandhi (2015) reported that the samples showed highest in linoleic acid - 47 to 57 per cent, palmitic acid - 19 to 15 percent, stearic acid - 3 to 4 per cent and oleic acid - 18 to 22 per cent. The percentage of oleic acid in cottonseed oil was more comparable to (corn oil 19-49%), (mustard oil 20-22%) and (soyabean oil 22-34%) (Nollet, 2004; Gandhi, 2015). Cottonseed contained cyclopropenoid fatty acids including of malvelic acid and sterculic acid, and these acids are considered as anti-nutritional which have been reported to reduce the gossypol levels in cottonseed and its kernel (Sunilkumar *et al.*, 2006; Dowd *et al.*, 2010).

Gossypol is a phenolic compound with the effective activity in antifertility and antioxidant, which is presented in cottonseed and its byproducts (Taghvaei *et al.*, 2015). The amount of gossypol content was determined in the selected cottonseed varieties by using UHPLC and their concentrations are presented in Fig. 1 and 2. The results showed that the gossypol content was ranging from 14.93 to

88.46mg/100g for the different samples. The varieties of cottonseed for SVPR 2, SVPR 3, SVPR 4, SVPR 5, K 2, K 9, K 11 and MCU 5 contained the gossypol concentration of 18.22, 17.35, 28.18, 47.67, 33.63, 88.46, 14.93 and 22.81mg/100g, respectively. It was clear that the gossypol content as presented in the samples (SVPR 2, SVPR 3, SVPR 4, K 2, K 11 and MCU 5) are under the limited intake level (450 ppm) set by USFDA regulation. Along with these varieties, SVPR 5 had met out the FAO/WHO regulation (600 ppm) to make better utilization of those cottonseeds and it's by-products for human consumption. The acetone-based extraction and UHPLC analysis of free gossypol content was accessible in a wide variation of selected cottonseed genotypes as 2.34 to 6.90mg/g (Gandhi, 2015). The above findings were correlated with non *Bacillus thuringiensis* varieties of cottonseed and its free gossypol content ranging between 1993.02 to 4139.7mg/kg (Karishma *et al.*, 2016) as accessed through high performance liquid chromatography technique.

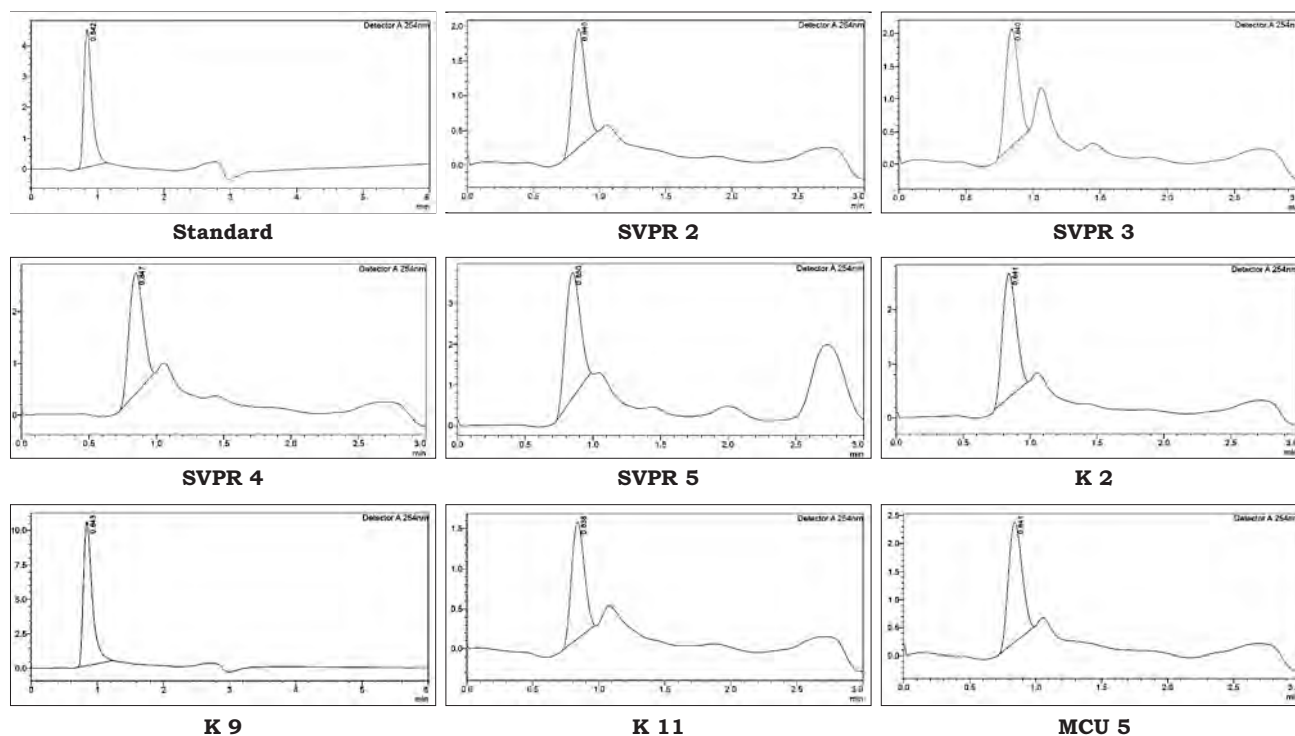


Fig. 1. Chromatogram for gossypol content in standard and selected cottonseed varieties

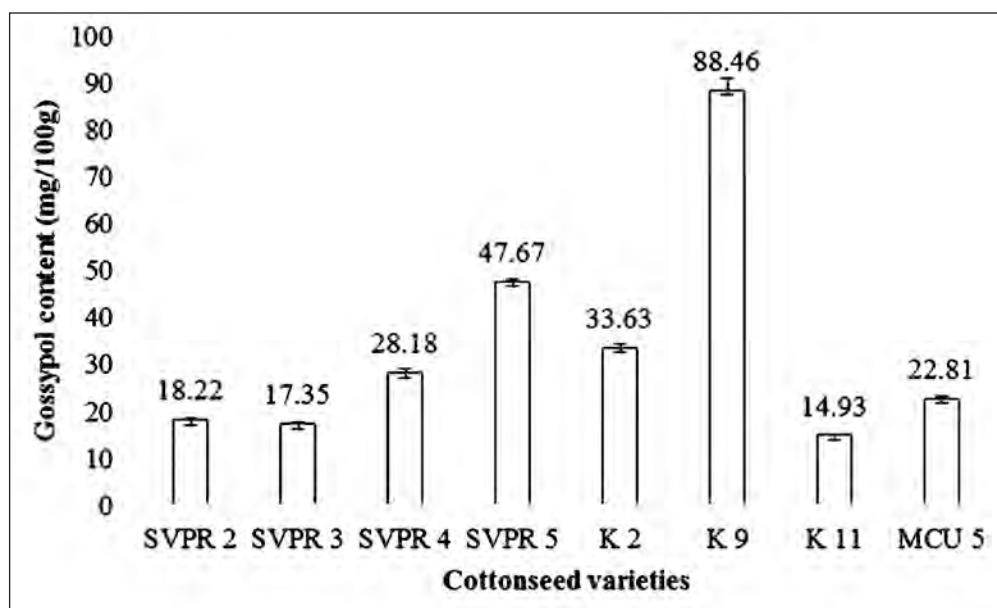


Fig. 2. Gossypol concentration in selected cottonseed varieties (mg/100g)

## CONCLUSION

The study showed that from the eight varieties of cottonseed samples selected from Tamil Nadu region, variety SVPR 2 had highly significant amount of crude protein and ash content, whereas the variety MCU 5 had maximum amount of crude lipid and energy content and also antioxidant activity compounds with much lower gossypol content for human consumption as set by USFDA and FAO/WHO. However, there is a gap from the harvesting to consumable stages. Due to lack of knowledge or awareness about the nutritional and health benefits of cottonseed among the public from the feed for animal, there is need to evaluate same findings and development of cottonseed based food products and food safety issues of the screened cottonseed varieties in better human nutrition and health.

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