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Research Article

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GC analysis of different parts of *Tecoma stans* (L.) Juss. ex Kunth for fatty acid composition

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ABSTRACT

The study evaluated the fatty acid profile of *Tecoma stans* (L.) Juss. ex Kunth plant parts. PUFAs were predominant in seed and leaf, except flower, which showed a higher amount of SFAs. Leaf exhibited the highest content of linolenic acid. cis-11,14-Eicosadienoic acid was the major fatty acid observed in leaf and seed. Another health beneficial n-6 FA observed in seed was cis-13,16-Docosadienoic acid. The C18:2n6c: C18:3n3c ratio of 4.75:1 confirms plant seed as an ideal source of n-6 FAs.

KEY WORDS: *Tecoma stans*, fatty acids, GC-FID, India

INTRODUCTION

Tecoma stans (L.) Juss. ex Kunth (Bignoniaceae), also known as *yellow-bells*, *yellow-elder*, *yellow trumpet bush*, *trumpet bush*, *ginger-thomas*, *esperanza*, *tronadora*, is native to the high altitude regions of South America and the drier habitats of North America. It has got naturalized in tropical and subtropical regions such as Africa, Asia, The Pacific Islands, and Australia. It is majorly used as an ornamental shrub with evergreen foliage, trumpet-shaped bright yellow, faintly fragrant bunchy flowers, and an abundance of fruits and seeds (CABI, 2020). As a medicinal plant, it is used traditionally for regulating high blood sugar levels; treating problems of the gastrointestinal tract, liver, kidney, eye, and skin, stimulating the immune system, and antidote against scorpion, snake; and rat bites (Winkelman, 1986; Irigoyen-Rascon & Paredes, 2015; Moe & Hlaing, 2019). Several pharmacological studies revealed its antioxidant, antidiabetic, cardioprotective, anticancer, anti-inflammatory, antiulcer, hepatoprotective, anti-arthritic, and antimicrobial actions (Aguilar-Santamaría *et al.*, 2009; Sbihi *et al.*, 2015; Taher *et al.*, 2016; Robinson *et al.*, 2017; Bakr *et al.*, 2019). Though the plant has played an essential role as a medicine for treating a broad spectrum of disease conditions, there is a paucity of information on its nutritional profiling. Up to now, 120 compounds have been identified and isolated from the plant, including monoterpene alkaloids, phenolic acids, flavonoids, carotenoids, phytosterols, volatile oils, and fatty acids (Sbihi *et al.*, 2015; Taher *et al.*, 2016). To date, data on the fatty acid profile of seed sample of the plant has been reported (Sbihi *et al.*, 2015). Besides this there are no reports on the comparative fatty acid composition of Tecoma plant parts. Thus our study aimed to determine the fatty acid profile of *Tecoma stans* plant parts grown in Puttaparthi, Andhra Pradesh, India.

MATERIALS AND METHODS

Plant Material and Chemicals

The plant materials such as the leaf, flower and pod were collected from the Institute Medicinal Garden, Sri Sathya Sai Institute of Higher Learning, Prashanthi Nilayam, Andhra Pradesh, India, during March 2019. The freshly collected samples were washed under running tap water and shade dried. Seeds were manually collected from each dried pod sample and shade dried for a day. The dried samples were pulverized to fine powder (150 mesh) and stored at 4°C. Herbarium sheets of the collected samples were authenticated by the Botanical Survey of India, Central regional centre, Allahabad, Ministry of environment, forest and climate change, India. A voucher specimen was also submitted at the Department of Biosciences, Sri Sathya Sai Institute of Higher Learning, Prashanthi Nilayam for future reference. The fatty acids methyl ester (FAME) reference standard mixture was purchased from Sigma-Aldrich, Germany. All the other chemicals used in the analysis were analytical grade and purchased from Himedia, India.

Fatty Acid Analysis

The fatty acids present in the dried samples were detected in the methylated oil samples, according to AOAC, 996.06 method (AOAC, 2005).

Extraction and methylation of fatty acids

Fat and fatty acids from the plant samples were extracted using the acid hydrolysis method. A weighed amount of sample was treated with 10 ml 8.3 M HCl and mixed well. They were kept in a water bath at 70 to 80°C for 40 minutes, along with moderate agitation and vortex mixing at every 10 minutes. After digestion, the samples were allowed to cool to room temperature (20 to 25°C), followed by ethanol addition.

Methylation

The obtained fat residue was dissolved in 2 to 3 ml of chloroform and diethyl ether, respectively. The samples were evaporated to dryness at 40°C under a nitrogen stream. To the evaporated samples added 2 ml 7 percent BF3 reagent and 1 ml toluene, followed by heating at 100°C for 45 minutes with gentle shaking every 10 minutes. The vials were cooled to room temperature (20 to 25°C), followed by the addition of 5 ml water, 1 ml hexane, and 1 g sodium sulfate and shaken for 1 minute. The layers were then allowed to separate, and the top layer contained fatty acid methyl esters (FAMEs). These were then injected onto the GC column for the analysis.

GC determination of FAMEs

The fatty acids present in the samples were detected with a gas chromatograph (Shimadzu) equipped with a hydrogen flame ionization detector (SP-2560, L x I.D. 100 m x 0.25 mm, df 0.20 µm, SIGMA, USA), split mode injector (split ratio, 200:1) along with oven temperature programming. The detector and injector temperatures were 225°C and 285°C, respectively. The carrier gas was helium (99.99 percent) at a flow rate of 0.75 ml/minute. The methylated oil samples (2 µl) were injected using the split mode. The percentage composition of the samples was calculated using the peak normalization method and expressed as g/100 g and percent of the total fat content dw.

Statistical Analysis

Duplicate determinations were done for fatty acid profile. The obtained results were expressed as mean values ± standard deviation. The obtained data was subjected to one-way Analysis of Variance (ANOVA). Significant difference between means was determined using Tukey-Kramer posthoc multiple comparison tests ($p<0.05$).

RESULTS AND DISCUSSION

The details of the chromatograms obtained on GC-FID analysis of *Tecoma stans* leaf, flower and seed are presented in Table 1 to 3 & Figure 1 to 3. As presented, seventeen fatty acids were observed in the samples, with polyunsaturated fatty acids (PUFAs) being predominant in seed and leaf, except flower, which showed a higher amount of saturated fatty acids (SFAs). The seed sample presented significantly ($p<0.05$) higher amount of total fat (16.19 g/100 g dw) and unsaturated fatty acids (13.76 g/100g dw) than leaf and flower. The maximum amount of SFAs was exhibited by flowers with the highest palmitic acid content, followed by myristic acid. All the samples yielded a higher % for long chain triglycerides (LCTs) when

Table 1: GC-FID analysis of *Tecoma stans* leaf

Peak	Retention time	Fatty acid	Area %	Area
1	13.1475	Caproic Acid (C6:0)	0.7137	11757
2	13.931	Caprylic Acid (C8:0)	0.3499	5764
3	18.711	Capric Acid (C10:0)	1.0167	16750
4	19.193	Lauric Acid (C12:0)	1.4743	24287
5	21.637	Myristic Acid (C14:0)	1.2783	21059
6	22.798	Myristoleic Acid (C14:1)	0.6466	10653
7	25.084	Pentadecanoic Acid (C15:0)	1.8944	31208
8	25.873	Cis-10-Pentadecanoic Acid (C15:1)	4.7515	78276
9	26.826	Palmitic Acid (C16:0)	17.2786	284651
10	28.093	Stearic Acid (C18:0)	1.2700	20923
11	30.934	Oleic Acid (C18:1n9c)	4.2773	70466
12	32.256	Linoleic Acid (C18:2n6c)	1.9979	32914
13	34.222	Linolenic Acid (C18:3n3)	16.9095	278571
14	34.946	Behenic Acid (C22:0)	1.5196	25034
15	36.484	cis-11,14-Eicosadienoic Acid (C20:2)	44.6216	735105
		Total	100.00	1647418

Table 2: Fatty acid GC-FID analysis of *Tecoma stans* flower

Peak	Retention time	Fatty acid	Area %	Area
1	13.098	Caproic Acid (C6:0)	0.5777	13473
2	14.418	Caprylic Acid (C8:0)	0.1811	4225
3	16.371	Capric Acid (C10:0)	0.1995	4652
4	19.209	Lauric Acid (C12:0)	1.0866	25342
5	22.805	Myristic Acid (C14:0)	6.2860	146599
6	26.862	Palmitic Acid (C16:0)	42.5346	991980
7	29.537	Palmitoleic Acid (C16:1)	3.0723	71652
8	30.948	Stearic Acid (C18:0)	10.5331	245650
9	32.267	Oleic Acid (C18:1n9c)	6.2438	145617
10	34.232	Linoleic Acid (C18:2n6c)	18.1395	423045
11	39.941	Linolenic Acid (C18:3n3)	0.9171	21388
12	36.467	cis-11,14-Eicosadienoic Acid (C20:2)	10.2286	238549
		Total	100.00	2332172

Table 3: Fatty acid GC-FID analysis of *Tecoma stans* seed

Peak	Retention time	Fatty acid	Area %	Area
1	13.103	Caproic Acid (C6:0)	0.3001	14987
2	26.846	Palmitic Acid (C16:0)	10.8647	542500
3	28.110	Palmitoleic Acid (C16:1)	0.2395	11961
4	30.978	Stearic Acid (C18:0)	3.8118	190334
5	32.299	Oleic Acid (C18:1n9c)	9.1889	458824
6	34.263	Linoleic Acid (C18:2n6c)	17.5971	878661
7	35.633	Linolenic Acid (C18:3n3)	3.7064	185067
8	36.536	cis-11,14-Eicosadienoic Acid (C20:2)	37.6110	1878000
9	37.991	cis-13,16-Docosadienoic Acid (C22:2)	16.6804	832889
		Total	100.00	4993223

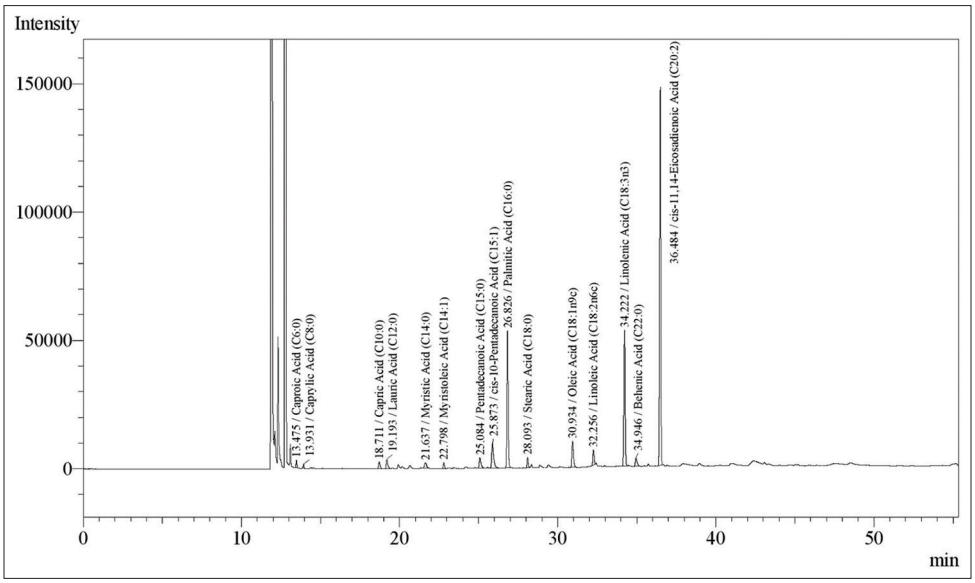

Figure 1: GC-FID chromatogram of *Tecoma stans* leaf

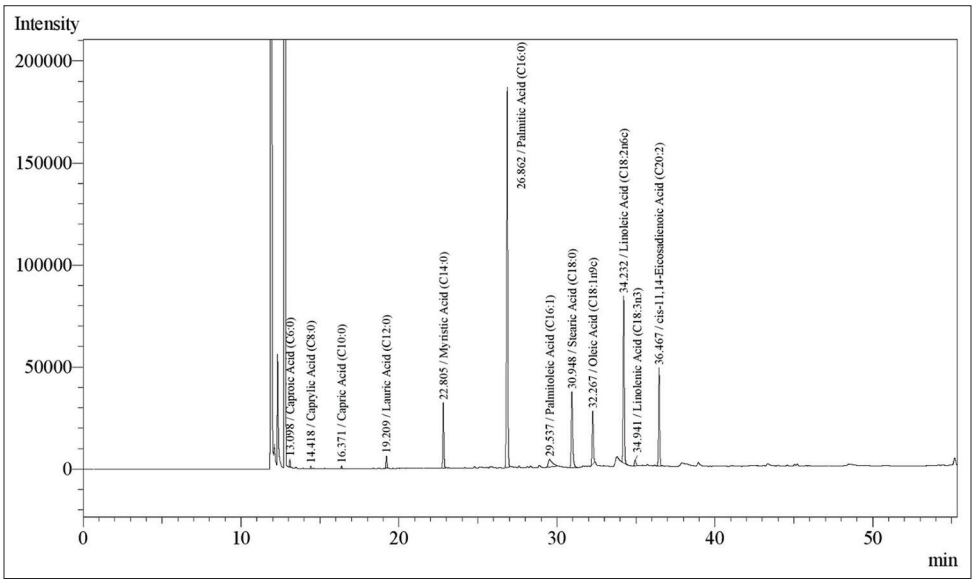

Figure 2: GC-FID chromatogram of *Tecoma stans* flower

compared with medium chain triglycerides (MCTs). Most notably, palmitic acid (C16:0) was the highest in all the samples, yielding 42.62, 17.21, and 10.87 % for flower, leaf, and seed of the total fat content, respectively. However, lower concentrations of remaining MCTs and LCTs such as caproic and stearic were observed in the samples. Likewise, small amounts of caprylic, capric, lauric, and myristic acid were detected in leaf and flower (Table 4). Additionally, the leaf sample showed the presence of pentadecanoic and behenic acids. Lower content of palmitic acid (6.09 %) and comparable level of stearic acid (4.12 %) have been reported in Tecoma seed (Shibi *et al.*, 2015).

UFAs are components of phospholipids in cell membranes that help maintain membrane fluidity, flexibility, and permeability. Humans lack Δ 12 and Δ 15 desaturases, which help introduce a cis double bond at the n-6 and n-3 position of the fatty acids; hence are termed as essential fatty acids. Their role in the formation and maintenance of healthy cell membranes; proper development and functioning of the brain and nervous system; maintenance of bone health; regulation of arterial pressure, lipid profile, blood viscosity, blood coagulation, immune and inflammatory responses is well-established (Youdim *et al.*, 2000; Saravanan *et al.*, 2010). Deficiency of these FAs can result in growth retardation, elevated cholesterol levels, underdeveloped brain and neural functioning, and dermal disorders (Connor *et al.*, 1992; Hamosh & Salem, 1998). In the present study, UFAs accounted for 84.99 % of the total fatty acids detected in the seed sample, which is in line with the reported % of 89.43 of total fat content (Sbihi *et al.*, 2015). The % obtained for PUFA was comparatively higher than MUFA in all the samples.

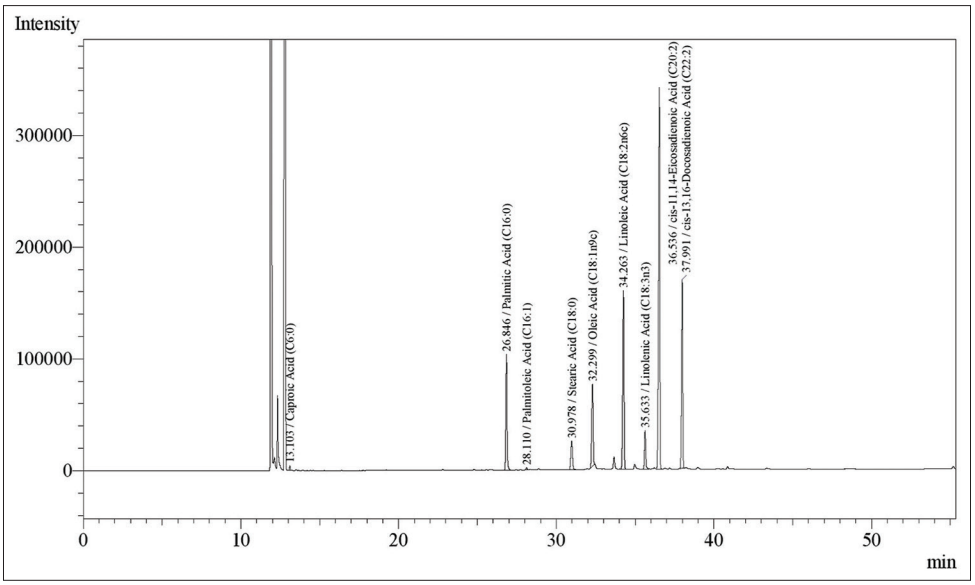

Figure 3: GC-FID chromatogram of *Tecoma stans* seed

Among MUFAs, C18:1n9c was the most abundant yielding 9.20 for seed, 6.24 for flower, and 4.30 for the leaf. Additionally, the leaf sample showed the presence of cis-10-Pentadecanoic and myristoleic acid. Palmitoleic acid was present in lower concentrations in flower and seed. Though the role of this fatty acid was well-known in the 1960s, it did not gain much attention then. Some of the recent pharmacological functions of this n-9 FA include increasing insulin sensitivity, improving lipid profile by decreasing its accumulation in the liver through its higher transcriptional activity, and modulating enzymes and cytokines. Passos *et al.*, (2016) concluded from their study that it suppresses lymphocyte activation and its proliferation via decreased inflammatory cytokines production. The plant leaf has potential in treating immune disorders; however, this requires clinical investigations to prove its role in enhancing immune function.

Linoleic acid (C18:2n6c) was the key UFA (% of total fat content) found in the flower (18.10) and seed (17.60). Lower linoleic acid content (11.48 %) in the seed was presented by Sbihi *et al.*, (2015). Linolenic acid (C18:3n3) was 16.89, 3.71, and 0.83 % of average fatty acid content for leaf, seed, and flower, respectively. Extremely high levels (%) of 23.56 for oleic and 45.47 for linolenic acid in seed originated from Riyadh, Saudi Arabia have been reported (Sbihi *et al.*, 2015). The authors also declared the presence of two additional fatty acids in the plant seed oil, namely stearidonic acid and γ-linolenic acid, which were not noticed in seed in our study.

Leaf and seed had cis-11,14-Eicosadienoic acid (C20:2n6) as the major fatty acid yielding 44.75 and 37.60 % of total fat content. It is an uncommon natural source of n-6 FA found in minimum quantities in animal tissues and is known to exert anti-inflammatory action by inhibiting the leukotriene B4 receptor's binding in pig neutrophil membrane (Yagaloff *et al.*, 1995). Seed sample alone showed the presence of cis-13,16 –Docosadienoic acid (C22:2n6). It is a very long chain n-6 FA that acts as a natural ligand for free fatty acid receptor 4 (FFAR4 or GPR120) and thereby activates these receptor's biological responses to regulate energy and bone metabolism and balance immune and neuronal function (Kimura *et al.*, 2020). Studies have shown that these receptors promote GLP-1 secretion, resulting in higher circulating levels of insulin in mice (Hirasawa *et al.*, 2005). Investigations have shown that this fatty acid strongly inhibits the secretion of ghrelin hormone by isolated mouse gastric cells indicating its use similar to leptin hormone, which is released from adipose tissues in response to the % of fat deposits in the body and accordingly regulate appetite and normalizes body weight (Lu *et al.*, 2012). Among the n-6 FAs analyzed in the seed sample, C22:2n6 was detected with a maximum % of total fat content. Since both n-6 FAs were present in maximum amounts and also have proved therapeutic effects, studies for evolving therapeutic drugs beneficial to human health can be initiated.

C18:2n6c: C18:3n3 ratio of 4:1 has proved to decrease overall mortality by 70 % (Simopoulos, 2002). However, an excessive amount of n-6 PUFAs, and a higher ratio of n-6:n-3 (16:1 or higher) have suggested triggering the risk of cardiovascular diseases, autoimmune diseases, and a few types of cancers and major depressive disorder whereas decreasing this ratio has shown suppressive effects (Rizos *et al.*, 2012; Husted & Bouzinova, 2016; Nindrea *et al.*, 2019). Among the samples studied, the calculated ratio for the plant seed was 4.75:1, which is slightly higher than the reported ratio (Simopoulos, 2002) and almost double the nutritionally recommended ratio of 2:1 (Simopoulos *et al.*, 1999). However, Simopoulos (2002) mentions that even a higher ratio of 5:1 has shown positive effects in the asthmatic population. Additionally, a ratio of 5:1 or lower than that has been proved to reduce pro-inflammatory cytokines and exhibit cardioprotective effect by regulating the serum cholesterol levels (Yang *et al.*, 2016). To maintain a balanced production of eicosanoids, an appropriate n-3:n-6 FAs

Table 4: Fatty acid composition of leaf, flower, and seed of *Tecoma stans* using GC-FID

Fatty acids	Amount (g/100g dw)		
	Leaf	Flower	Seed
Caproic acid (C6:0)	0.04±0.001^{b} (0.69)*	0.03±0.002^{b} (0.62)	0.05±0.005^{a} (0.31)
Caprylic acid (C8:0)	0.02±0.002^{a} (0.34)	0.01±0.006^{b} (0.21)	nd
Capric acid (C10:0)	0.06±0.010^{a} (1.03)	0.01±0.006^{b} (0.21)	nd
Lauric acid (C12:0)	0.09±0.004^{a} (1.55)	0.05±0.008^{b} (1.04)	nd
Myristic acid (C14:0)	0.08±0.002^{b} (1.38)	0.30±0.007^{a} (6.24)	nd
Myristoleic acid (C14:1)	0.04±0.003 (0.69)	nd	nd
Pentadecanoic acid (C15:0)	0.11±0.005 (1.89)	nd	nd
cis-10-Pentadecanoic acid (C15:1)	0.28±0.004 (4.82)	nd	nd
Palmitic acid (C16:0)	1.00±0.003^{c} (17.21)	2.05±0.038^{a} (42.62)	1.76±0.040^{b} (10.87)
Palmitoleic acid (C16:1)	nd	0.15±0.001^{a} (3.12)	0.04±0.002^{b} (0.25)
Stearic acid (C18:0)	0.08±0.005^{c} (1.38)	0.51±0.018^{b} (10.60)	0.62±0.033^{a} (3.83)
Oleic acid (C18:1n9c)	0.25±0.000^{b} (4.30)	0.30±0.029^{b} (6.24)	1.49±0.033^{a} (9.20)
Linoleic acid (C18:2n6c)	0.12±0.010^{c} (2.07)	0.87±0.017^{b} (18.10)	2.85±0.042^{a} (17.60)
Linolenic acid (C18:3n3c)	0.98±0.042^{a} (16.89)	0.04±0.001^{c} (0.83)	0.60±0.016^{b} (3.71)
cis-11,14-Eicosadienoic acid (C20:2n6)	2.60±0.020^{b} (44.75)	0.49±0.000^{c} (10.19)	6.09±0.074^{a} (37.62)
Behenic acid (C22:0)	0.09±0.001 (1.55)	nd	nd
cis-13,16-Docosadienoic acid (C22:2n6)	nd	nd	2.70±0.047 (16.68)
MCTs	0.21±0.004^{a} (3.61)	0.10±0.005^{b} (2.08)	0.050±0.005^{c} (0.31)
LCTs	1.36±0.012^{c} (23.41)	2.86±0.056^{a} (59.46)	2.38±0.069^{b} (14.70)
Total SFAs	1.57±0.033^{c} (27.02)	2.96±0.084^{a} (61.54)	2.43±0.078^{b} (15.01)
MUFAs	0.57±0.006^{b} (9.81)	0.45±0.027^{b} (9.35)	1.53±0.035^{a} (9.45)
PUFAs	3.70±0.020^{b} (63.68)	1.40±0.020^{c} (29.11)	12.23±0.065^{a} (75.54)
n-3 FAs	0.98±0.017^{a} (16.86)	0.04±0.001^{c} (0.83)	0.60±0.016^{b} (3.71)
n-6 FAs	2.72±0.020^{b} (46.82)	1.36±0.020^{c} (28.27)	11.63±0.060^{a} (17.83)
Total UFAs	4.27±0.012^{b} (73.49)	1.85±0.046^{c} (38.46)	13.76±0.070^{a} (84.99)
Total fat content	5.81±0.045^{b}	4.81±0.130^{c}	16.19±0.148^{a}
C18:3n3c: C18:2n6c	8.17:1	0.046:1	0.21:1
C18:2n6c: C18:3n3c	0.12:1	21.75:1	4.75:1
UFAs : SFAs	2.72:1	0.63:1	5.67:1

Values are expressed as mean ± SD of duplicate determinations. Means in each row followed by different superscripts are significantly different ($p<0.05$); * Values in parentheses present % of total fat content; nd- not detected; MCTs- Medium chain triglycerides; LCTs- Long chain triglycerides; SFAs- Saturated fatty acids; UFAs- Unsaturated fatty acids; MUFAs- Monounsaturated fatty acids; PUFAs- Polyunsaturated fatty acids, n-3 FAs- omega3 fatty acids; n-6 FAs – omega 6 fatty acids

ratio of 2:1-3:1 has been recommended by Simopoulos (2003). None of the studied samples exhibited ratios within this range.

CONCLUSION

To our information, the present work marks the first comparative study of the fatty acid profile of *Tecoma stans* plant parts. The obtained data shows that these parts are an abundant source of health beneficial fatty acids. Among the samples studied, seed presented a maximum level of n-6 FAs whereas leaf was a good source of n-3 FAs. As a significant source of UFAs, the plant seed and leaf can yield substantial benefits to vegetarians and other populations where consumption of fish oil is either low or completely neglected due to food taboos or personal likes and dislikes. Like other vegetable oils, the seed oil has a reasonable n-6:n-3 ratio. Thus, it can be explored as a new plant based source of oil; however, its utilization as edible oil or in pharmaceutical applications needs clinical investigations.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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