GRASAS Y ACEITES 74 (2)
April-June 2023, e499
ISSN-L: 0017-3495
https://doi.org/10.3989/gya.0907212

GC/MS quantification of individual fatty acids of selected green leafy vegetable foliage and their biodiesel attributes

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Submitted: 28 September 2021; Accepted: 04 July 2022; Published online: 25 May 2023

SUMMARY: The current demand for edible vegetable oil is increasing worldwide, and the development of new sources of high-quality vegetable edible oil is an essential task. There is also a huge demand for biodiesel in domestic and industrial applications, and foliage oils could be a good source for diesel applications. The current study aimed at the identification and quantification of fatty acids from commonly consumed green leafy vegetables (GLVs) viz., *Hibiscus cannabinus, Hibiscus sabdariffa, Basella alba, Basella rubra,* and *Rumex vesicarius* and to calculate the biodiesel attributes of the oil. The total oil content was ascertained as the highest in *R. vesicarius* foliage (3.91 ± 0.27 g/100 g dry leaf powder). GC/MS chromatographic investigation identified 9,12,15-octadecatrienoic acid as a significant compound followed by hexadecanoic acid. In *Hibiscus* spp. C18:3 (49.3 µmol % and 50.4 µmol %) was recorded to be the most noteworthy followed by C16:0 (23.2 µmol % and 21 µmol %) in *H. cannabinus* and *H. sabdariffa,* respectively. The GLVs foliage-fatty acid biodiesel attributes were additionally assessed through an empirical formula. Consequently, the overall examined results will be helpful for the investigation of these oils as vegetable oil for human consumption and biodiesel applications.

KEYWORDS**:** *Biodiesel properties; Fatty acids; Green leafy vegetables; Total oil*

RESUMEN: ***Cuantificación por GC/MS de la composición de ácidos grasos del follaje de hortalizas de hoja verde seleccionadas y sus atributos para biodiesel.*** La demanda actual de aceite vegetal comestible está aumentando en todo el mundo, y el desarrollo de nuevas fuentes de aceite vegetal comestible de alta calidad es una tarea esencial. También existe una gran demanda de biodiesel para aplicaciones domésticas e industriales, así, los aceites de follaje podrían ser una buena fuente para estas aplicaciones. El presente estudio tuvo como objetivo la identificación y cuantificación de ácidos grasos de vegetales de hoja verde (GLV) de consumo común, como, *Hibiscus cannabinus*, *Hibiscus sabdariffa*, *Basella alba*, *Basella rubra* y *Rumex vesicarius*, y determinar los atributos para el biodiesel de los aceite. El contenido total de aceite más alto se obtuvo para el follaje de *R. vesicarius* (3,91 ± 0,27 g/100 g de polvo de hojas secas). La determinación cromatográfica, GC/MS, identificó al ácido 9,12,15-octadecatrienoico como el ácido mayoritario, seguido del ácido hexadecanoico. En *Hibiscus* spp. el ácido C18:3 fue el mayoritario (49,3 µmol % y 50,4 µmol %), seguido de C16:0 (23,2 µmol % y 21 µmol %) en *H. cannabinus* y *H. sabdariffa*, respectivamente. Los características para el biodiesel de los ácidos grasos de follaje de GLV también se evaluaron empíricamente. En consecuencia, los resultados generales obtenidos serán útiles para investigaciones de estos aceites como aceites vegetales para uso humano y aplicaciones de biodiesel.

PALABRAS CLAVE: *Aceite total; Ácidos grasos; Hortalizas de hoja verde; Propiedades del biodiésel*

Citation/Cómo citar este artículo: Kumar SS, Manasa V, Madhubalaji CK, Tumaney AW, Giridhar P. 2023. GC/MS quantification of individual fatty acids of selected green leafy vegetable foliage and their biodiesel attributes. *Grasas Aceites* **74** (2), e499. https://doi.org/10.3989/gya.0907212

1. INTRODUCTION

The current demand for edible vegetable oil is increasing worldwide (Wu *et al*., 2020). An enormous part of oils and fats are obtained from plants (leaves, seeds, and fruits), and most of them are used for human or industrial use (Diemeleou *et al*., 2014). Overall, 75% of vegetable oils are in a liquid state which is utilized in the food industry for frying, canning, and the preparation of margarine and emulsions (Salas *et al*., 2009). There are notable, well-known vegetable oils, for example, palm, peanut, coconut, and sunflower oils and so on (Gunstone, 2011).

Sustainable and affordable plant sources as alternate sources of renewable energy are gaining greater significance from an environmental perspective (Valenga *et al*., 2019). The post-harvest agricultural residues and seeds of a good number of plants have been explored in this regard (Kumar *et al*., 2020). However, few reports show the production of bio-oils from vegetative parts especially the foliage of plants which can be generated without difficulty (Hoseini *et al*., 2019). Biodiesel is a renewable fuel that is a sustainable option to meet energy demands compared to petroleum-derived diesel. Biodiesel will reduce the environmental effects of burning fossil fuels (Valenga *et al*., 2019).

Fatty acids are one of the main ingredients in edible oil, and many nutritional properties are related to them. The fatty acid composition, trans-esterification, long-chain saturated factor, and the degree of unsaturation provide information about the biodiesel properties of oils (de Freitas *et al*., 2019). These parameters could be used to estimate the cetane number, cold filter plugging point, iodine value, and other parameters of biodiesel (de Freitas *et al*., 2019). As these selected unexplored edible GLVs foliage oils are more affordable compared to exorbitant vegetable oils which are accessible commercially. This could be conceived as promising selective post-harvest foliage feedstock for biodiesel production because these GLVs are richly accessible all over the globe. In order to fulfil this expanding need for oil-yielding vegetable sources, we chose GLVs such as *Hibiscus cannabinus*, *Hibiscus sabdariffa*, *Basella alba*, *Basella rubra* and *Rumex vesicarius* for the GC/MS characterization of fatty acid composition. In addition, based on the fatty acid profiling, the biodiesel properties of the GLVs were calculated using empirical formulas for commercial exploration. To our knowledge, this is the first report on the forecast of biodiesel properties for the five chosen GLV foliage.

The genus Hibiscus contains more than 300 species of angiosperms (Kim *et al*., 2019). *Hibiscus cannabinus* and *Hibiscus sabdariffa* (Malvaceae), commonly known as kenaf and roselle are the two most common species. They are industrially important fibrous plants and are also known worldwide in the food and nutraceutical industries (Jin *et al*., 2013). In Ayurveda, the leaves are utilized for bilious, blood, diabetes, hacks, and throat issues (Jin *et al*., 2013). They are used in processed foods, as a flavoring agent, or in cold or hot beverages as an herbal remedy for hyperlipidemia, and hypertension (Paraíso *et al*., 2020). The oil extracted from seeds of *Hibiscus* spp. showed two unusual fatty acids such as di-hydro sterculic acid, and vernolic acids (Wang *et al*., 2012). The seeds of the plant contain omega-3-fatty acids, phenolics, sterols, and so forth (Wang *et al*., 2012).

In *Basella* spp. there are two popular varieties such as *Basella rubra* (red leaf, stem, and fruits) and *Basella alba* (green leaf and stem) belonging to Basellaceae (Kumar *et al*., 2018). The nutritional and antioxidant potentials of the leaf extracts of *Basella* spp have been reported (Kumar *et al*., 2015a). In addition, the nutraceutical possibilities of *B. rubra* fruit extracts on human cervical cancer cells (SiHa) have likewise been illustrated (Kumar *et al*., 2015b). Recently, the *B. rubra* seeds have been reported to contain 33% total oil with a good amount of nutraceutical compounds (phytosterols, tocopherols, polyphenols, and oryzanol) and 1% squalene has been reported (Kumar *et al*., 2020). The biochemical profile of *B. alba* seed oils relates to yield, specific gravity, color, and fatty acid composition (Diemeleou *et al*., 2014).

Rumex vesicarius L. (Polygonaceae) is referred to as blister sorrel, and rosy dock in English, and in Indian vernacular dialects it is well-known as chooka, chukka Kura, or chukki soppu. The leaves of the herb contain carotenoids, vitamin C, proteins, lipids, organic acids and minerals (Alfawaz *et al*., 2006). In folklore medicine, it is used for pain-relieving, as an astringent, hepatoprotective agent, remedy for tumours, and scurvy (Mostafa, 2014). Nonetheless, in all the chosen GLVs the information on the oil content, GC/MS fatty acid profiles, and biodiesel properties of the foliage had not been reported to date.

2. MATERIALS AND METHODS

2.1. Chemicals

HPLC grade hexane and anhydrous sodium sulfate were obtained from Sisco Research Laboratory (Mumbai, India) and Boron trifluoride solution (BF_3) was procured from Sigma Aldrich, Bangalore, India. All other chemicals used were of analytical grade.

2.2. Source of the fresh foliage material

The seeds of all five selected GLVs such as *Hibiscus cannabinus* L*., Hibiscus sabdariffa* L*., Basella alba* L*., Basella rubra* L., and *Rumex vesicarius* L., were collected from the local markets of Tirupati, Andhra Pradesh, India. The seeds were sown in micropots containing sand:soil:compost (1:1:1) for seed germination, maintained at a controlled temperature and relative humidity under greenhouse conditions (Figure 1). The botanical confirmation of the selected plant was confirmed, and herbarium specimen was deposited at the Herbarium deposition center of the Department of Botany, University of Mysore, Mysore (Ref. No.02.08.13). The leaves of the mature plants were collected separately and dried in a hot air oven set at 55 °C for complete dryness. The dried leaf samples were made to a coarse powder using a mixer grinder (Maharaja Whiteline Perfect W&R 500 Mixer grinder) for 3 ± 0.2 min at high speed to maintain a uniform particle size and stored in polythene airtight bags until further use.

2.3. Determination of total oil content

The total oil was extracted from the above-prepared leaf powder samples with hexane in a Soxhlet extractor, wherein the samples were subjected to heating a round-bottom flask containing boiling chips on a water bath set at 60 ± 5 °C for about 8 h. The obtained hexane-oil mixture was filtered using Whatman filter paper no. 1 containing anhydrous sodium sulfate and the filtrate was evaporated under reduced pressure in a pre-weighed round-bottom flask in a rotary evaporator (Hei-VAP Advantage, Heidolph Instrument GmbH & Co. KG, Schwabach, Germany). The flasks were kept for one min in a hot air oven set at 100 °C to evaporate the leftover hexane residue, cooled in a desiccator, and the difference in weight of the flask was measured using a sensitive balance to determine the total oil content (AOCS, 2003). The obtained oil samples were stored at -20 °C in screw-cap vials until further use.

Figure 1. The five selected GLVs cultivated under greenhouse conditions a. *H. sabdariffa*, b. *H. cannabinus*, c. *B. rubra*, d. *B. alba,* and e. *R. vesicarius*. Scale bar is 20 cm.

2.4. Fatty acid analysis by GC/MS

The fatty acid methyl esters (FAME) were prepared for the five selected foliage oil samples in triplicate by trans-esterification (Figure 2), according to the AOCS Official Method (AOCS, 2003). Briefly, for 100 mg of oil sample, 1 mL of BF_3 methanol was added and kept in a water bath for 30 min at 60 °C. The tubes were immediately transferred into the ice bath for 5 min, and 1 mL hexane was added, followed by 1 mL distilled water and the tubes were vortexed for complete mixing. The reaction mixture tubes were kept aside for layer separation, and the upper layer was collected in a tube containing anhydrous sodium sulfate for the removal of moisture. Finally, the undisturbed top methyl ester layer free of water moieties and residual particles was transferred to GC vials for GC/MS analyses. 1 mg/mL heptadecanoic acid (C17:0) was added as the internal standard. Before GC/MS analysis, 0.2 µm nylon membrane filtered samples were used for analysis.

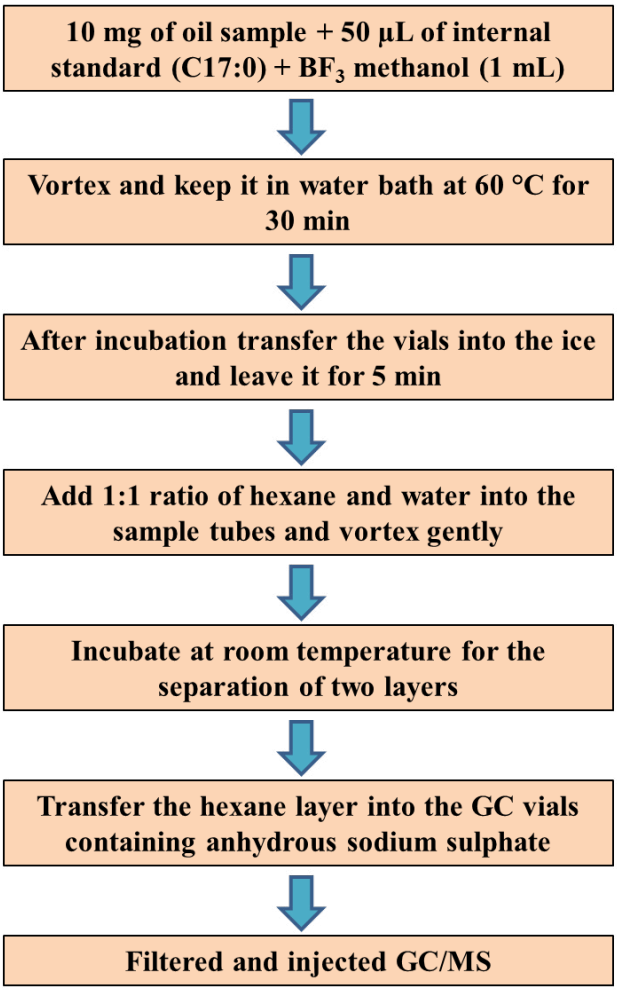

Figure 2. Block diagram representing the trans-esterification process of fatty acids. BF_3 – Boron trifluoride.

The GC/MS analysis was performed using an Agilent HP-7890B chromatograph connected directly to a 5977 inert mass spectrometer (Agilent Technologies, Milan, Italy), with GC column, DB-23 (60 m 0.25 mm I.D 0.25 mm film thickness). The analyses were performed in splitless mode (0.5 min), at 25 °C inlet temperature, with helium as carrier gas at a flow rate of 1 mL/min. The temperature was programmed at 10 °C/min to 300 °C, and then isothermal at 300 °C for 5 min. The MS detector was operated in electron ionization (EI) mode (70 eV, 200 mA), in full-scan mode (*m/z* 40-400), and also in selected–ion monitoring (SIM) mode (ions at *m/z* 127, 140, and 256 for heptadecanoic acid as the internal standard). The transfer line was set at 290 °C, and the solvent delay was set at 3 min.

2.5. Determination of biodiesel characteristics of the five selected foliage oils

2.5.1. Thermo-physical properties

Thermo-physical properties of the extracted oils for biodiesel properties such as density, kinematic viscosity and higher heating values were determined by using the modelled empirical equations (Srinivasan and Jambulingam, 2019):

Density (kg/m^3) = 881.86 - (0.07 × M) + (11.91 × D)

Kinematic viscosity (×10^{-6} m^2/s) = -5.59 + (0.04 × M) - (0.78 × D)

Higher heating value (MJ/kg) = 25.7+ (0.057 × M)-(3.16 × D)

Where, D = Number of Double bonds and M = Molecular Mass.

2.5.2. Viscosity determination

The absolute viscosity of the oils was determined by using the following relationship as reported by (Igbum *et al*., 2013):

$$\text{Viscosity } (\ln\eta) = -4.80 + 2525.93 \times \left(\frac{1}{T}\right) + 1.61 \times \left(\frac{(SV^2)}{T^2}\right) - (101.06 \times (IV^2) \times 10^{(-7)})$$

Where, T=Temperature, SV=Saponification value, IV=Iodine value, η=Viscosity and all the vales are constants.

2.5.3. Determination of ester content

The ester content of the oils was determined experimentally, and by using empirical correlations, the major biodiesel properties such as saponification value (SV), cetane number (CN), iodine value (IV) and degree of unsaturation (DU) were calculated (Madhubalaji *et al*., 2020):

$$SV = \sum \frac{(560 \times N)}{M}$$

$$CN = 46.3 + \left(\frac{5458}{SV}\right) - (0.225 \times IV)$$

$$IV = \sum \frac{(254 \times D \times N)}{M}$$

$$DU = MUFA + (2 \times PUFA)$$

Where, SV – saponification value, CN – cetane number, IV – iodine value, D – number of double bonds, M – molecular mass, N – Percentage of each fatty acid, MUFA – monounsaturated fatty acids and PUFA – polyunsaturated fatty acids.

2.5.4. Biodiesel cold flow properties

Long-Chain Saturated Factor (LCSF) and Cold-Filter Plugging Point (CFPP) were also calculated using empirical correlations (Madhubalaji *et al*., 2020):

LCSF (°C) = (0.1 × C16:0 + 0.5 × C18:0 + 1 × C20:0) + 1.5 × (C22:0 + 2 × C24:0)

CFPP (°C) = 3.1417 × LCSF – 16.477

2.5.5. Heat of combustion

The gross calorific value H or heat content of vegetable oils is the quantity of heat evolved when one mole of oil is burnt to carbon dioxide (CO_2) and water (H_2O) may be obtained from its structure indices using the relationship:

H = 47.645 – [4.187(IV) + 38.31(SV)] kJ/kg

2.6. Carbon, Hydrogen, Nitrogen and Sulphur (CHNS) contents

The selected five oil samples were analyzed for CHNS content using an Elemental Analyzer (Vario EL III, Germany) where sulphanilic acid was used as a standard compound. Briefly, 5.0-10.0 mg of oil sample were filled in a tin capsule and weighed using an electronic balance (Sartorius). The percentage of CHNS content was determined (Sekhar *et al*., 2018).

2.7. Statistical analysis

All the results are presented in the form of mean ± S.D of three replicates. Data were subjected to one-way ANOVA followed by post hoc Duncan's Multiple Range Test (DMRT) using SPSS 17 (SPSS Inc., Chicago, IL, USA) for determining significance at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Total oil content

The *R. vesicarius* showed the highest (3.91 ± 0.27) oil content; whereas the least was seen in *B. alba* (1.10 ± 0.08) and *B. rubra* (1.40 ± 0.10) in g/100 g of dry weight of leaves (DW). There was not much difference in the oil content found in *Hibiscus* spp. and *H. sabdariffa* contained 1.61 ± 0.14, and *H. cannabinus* contained 1.40 ± 0.12 g/100 g oil on DW. The reported 19% oil content in *H. sabdariffa* seeds contained a good amount of lipid-soluble antioxidant compounds such as γ-tocopherols at 0.2% (Mohamed *et al*., 2007). Similarly, the fatty acid profile of the *B. rubra* fruit pulp showed a total oil content of 1.38% with palmitic acid as the major fatty acid (Kumar *et al*., 2016).

3.2. Fatty acid profile

GC/MS chromatographic analysis revealed different compounds from the FAME prepared from the selected GLVs. Based on the retention times, molecular formulas, and molecular weights, the area percentage of each compound was presented (Table 1). Among the identified compounds, 9,12,15-octadecatrienoic acid (z,z,z) methyl ester followed by hexadecanoic acid methyl ester with 38.61 and 14.11% peak area of *H. cannabinus* and *H. sabdariffa* foliage were recorded, respectively. In *B. alba* samples, 9,12-octadecadienoic acid (z,z) methyl ester, followed by hexadecanoic acid methyl ester at 23.55 and 21.16%, respectively, were prominent. Nonetheless, in *B. rubra*, the hexadecanoic acid methyl ester was discovered to be the most elevated, followed by 9-Octadecadienoic acid (z) methyl ester with 39.73%. Essentially, in *R. vesicarius*, the 9,12,15-octadecatrienoic acid (z,z,z) methyl ester was the most

Table 1. Various compounds detected by GC/MS in the five selected GLV foliage oil samples.

Compound name	Molecular Formula	Molecular weight	*H. cannabinus*		*H. sabdariffa*		*B alba*		*B. rubra*		*R. vesicarius*	
			+/-	Area (%)	+/-	Area (%)	+/-	Area (%)	+/-	Area (%)	+/-	Area (%)
Dodecanoic acid methyl ester	$C_{13}H_{26}O_2$	214	-	-	-	-	+	0.25 ± 0.01	+	0.79 ± 0.04	-	-
Phytol acetate	$C_{22}H_{42}O_2$	338	+	0.53 ± 0.02	+	0.40 ± 0.01	+	0.47 ± 0.02	-	-	+	0.53 ± 0.02
Phytol acetate	$C_{22}H_{42}O_2$	338	+	0.81 ± 0.06	+	0.62 ± 0.04	+	0.64 ± 0.02	-	-	+	0.77 ± 0.03
2(3H)-Furanone-dihydro-5-pentyl	$C_9H_{16}O_2$	156	+	1.69 ± 0.14	+	1.34 ± 0.08	+	1.31 ± 0.05	-	-	+	1.45 ± 0.07
Phytol acetate	$C_{22}H_{42}O_2$	338	+	0.40 ± 0.02	-	-	+	0.40 ± 0.02	+	1.74 ± 0.03	+	0.40 ± 0.01
Phosphoric acid monododecyl ester	$C_{12}H_{27}O_4$	266	+	0.67 ± 0.04	+	0.46 ± 0.05	+	0.50 ± 0.04	-	-	+	0.60 ± 0.02
Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	+	0.84 ± 0.06	+	0.52 ± 0.04	+	0.58 ± 0.02	+	1.48 ± 0.11	+	0.70 ± 0.02
Phytol acetate	$C_{22}H_{42}O_2$	338	+	4.10 ± 0.168	+	3.15 ± 0.21	+	2.97 ± 0.38	+	1.47 ± 0.12	+	3.43 ± 0.12
3,3,5,5-Tetramethyl cyclohexanol	$C_{10}H_{20}O$	156	+	9.11 ± 0.34	+	7.28 ± 0.43	+	6.65 ± 0.24	+	5.15 ± 0.24	+	7.22 ± 0.22
Hexadecanoic acid methyl ester	$C_{17}H_{32}O_2$	270	+	16.81 ± 0.53	+	14.11 ± 1.32	+	21.16 ± 1.68	+	39.73 ± 2.68	+	18.22 ± 1.21
9-Hexadecenoic acid methyl ester	$C_{17}H_{32}O_2$	268	+	2.58 ± 0.15	+	2.48 ± 0.30	+	0.67 ± 0.04	+	1.44 ± 0.12	+	1.79 ± 0.14
Heptadecanoic acid methyl ester	$C_{18}H_{36}O_2$	284	+	3.68 ± 0.28	+	3.17 ± 0.38	+	3.06 ± 0.12	+	4.05 ± 0.21	+	1.25 ± 0.11
Nonanedioxic acid dimethyl ester	$C_{11}H_{20}O_4$	216	-	-	-	-	-	-	+	1.13 ± 0.02	-	-
2-Ethyl butyric acid heptadecyl ester	$C_{23}H_{46}O_2$	354	+	1.19 ± 0.24	+	0.89 ± 0.04	+	0.82 ± 0.08	-	-	+	1.07 ± 0.05
Methyl stearate	$C_{19}H_{38}O_2$	298	+	2.07 ± 0.11	+	1.82 ± 0.22	+	3.41 ± 0.26	+	6.04 ± 0.25	+	1.64 ± 0.06
9-Octadecadienoic acid(z) methyl ester	$C_{19}H_{36}O_2$	296	+	2.71 ± 0.22	+	2.45 ± 0.13	+	8.79 ± 0.89	+	12.80 ± 0.62	+	2.74 ± 0.11
11-Octadecanoic acid methyl ester	$C_{19}H_{36}O_2$	296	-	-	-	-	-	-	+	0.60 ± 0.02	-	-
12-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	-	-	-	-	-	-	-	-	+	0.47 ± 0.02
9,12-Octadecadienoic acid(z,z) methyl ester	$C_{19}H_{34}O_2$	296	+	12.16 ± 0.86	+	12.04 ± 1.02	+	23.55 ± 2.43	+	8.03 ± 0.21	+	15.65 ± 0.21
9,12,15-Octadecatrienoic acid(z,z,z) methyl ester	$C_{19}H_{32}O_2$	292	+	38.61 ± 2.58	+	36.59 ± 2.39	+	20.44 ± 1.68	+	7.52 ± 0.14	+	38.90 ± 2.04
Docosanoic acid methyl ester	$C_{22}H_{44}O_2$	340	+	0.53 ± 0.02	-	-	+	0.47 ± 0.02	+	0.99 ± 0.09	+	0.98 ± 0.09
Nonacosane	$C_{29}H_{60}$	408	+	1.10 ± 0.04	+	0.71 ± 0.04	-	-	-	-	-	-
Ethyl isoallocholate	$C_{22}H_{44}O_2$	436	-	-	-	-	-	-	+	0.89 ± 0.06	+	0.57 ± 0.01
Tetracosanoic acid methyl ester	$C_{22}H_{50}O_2$	382	+	0.42 ± 0.03	-	-	+	0.92 ± 0.11	+	1.84 ± 0.11	-	-
Squalene	$C_{30}H_{50}$	410	-	-	+	1.83 ± 0.14	+	1.20 ± 0.08	-	-	-	-
Octacosane	$C_{28}H_{58}$	394	-	-	+	10.15 ± 1.03	-	-	-	-	-	-
2,2,4-Trimethyl-1,3-(3,3,12,16-Tetramethyl heptadeca-3,7,14,15-tetraenyl)-cyclohexanol	$C_{30}H_{52}O$	428	-	-	-	-	-	-	+	1.00 ± 0.04	-	-
6,9,12-Octadecatrienoic acid methyl ester	$C_{19}H_{32}O_2$	292	-	-	-	-	-	-	+	1.16 ± 0.05	-	-
Octadecane, 3-ethyl-5-(2-ethyl butyl)	$C_{26}H_{54}$	366	-	-	-	-	+	1.74 ± 0.22	+	2.18 ± 0.12	+	1.61 ± 0.08

All values represented are mean ± SD of three replicates analysed. +/- indicate the presence / absence of compounds analyzed by GC/MS.

noteworthy (38.90%), followed by hexadecenoic acid methyl ester (18.22%) individually.

The quantification profile of the fatty acids of the selected foliage showed that in *Hibiscus* spp. C18:3 (49.3 µmol % and 50.4 µmol %) was recorded to be the highest, followed by C16:0 (23.2 µmol % and 21 µmol %) in *H. cannabinus* and *H. sabdariffa,* respectively. However, C18:2 (28.2 µmol %) was recorded to be the highest followed by C16:0 (27.5 µmol %) in *B. alba;* whereas *B. rubra* recorded C16:0 (49.9 µmol %) as the highest fatty acid followed by C18:1 Cis 12 (15.3 µmol %). Similarly, C18:3 was the major fatty acid (47.2 µmol %) followed by C16:0 (24 µmol %) in *R. vesicari-*

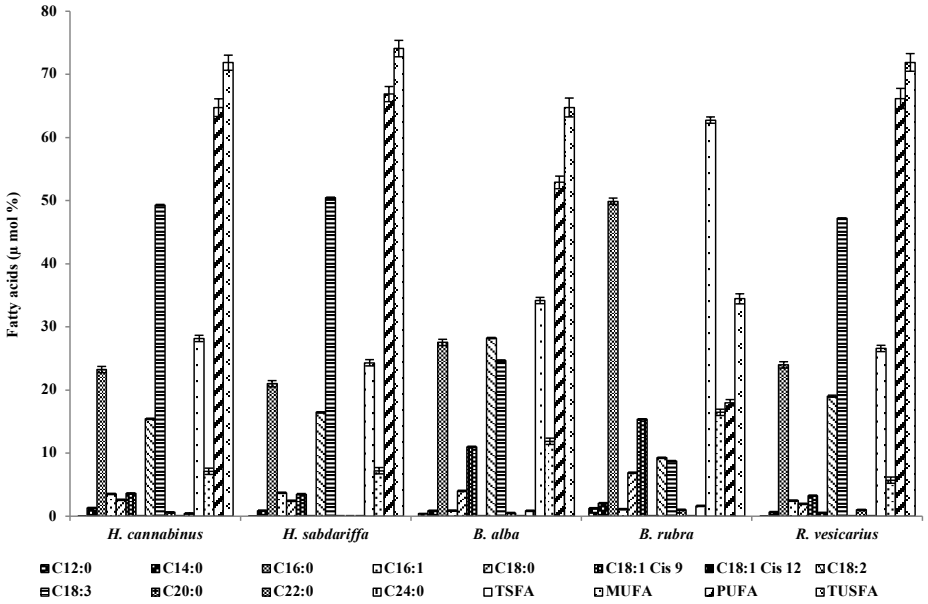

FIGURE 3. Fatty acid profiles of the five selected GLVs. Values are mean ± SD of three replicates analyzed. TSFA - Total saturated fatty acid, MUFA - Mono unsaturated fatty acid, PUFA - Poly unsaturated fatty acid and TUSFA - Total unsaturated fatty acid

us (Figure 3). With the exception of *B. rubra,* every single other extracted sample showed the highest total unsaturated fatty acid composition (TUSFA) above 65% with the significant composition of polyunsaturated fatty acids (PUFA) above 52% individually (Figure 3). This higher PUFA may add to the flexibility, fluidity and selective permeability of cellular membranes which will beneficially reduce cardiovascular ailments (Das, 2006). The structure of rice grain oil and its higher free fatty acid values were in connection with the investigations on vegetable oils (Gopala Krishna *et al*., 2006). The higher content of C18:3 in *Hibiscus* spp and *R. vesicarius* oil could be beneficial for the use of these oils in the cosmetic industry for the dehydration of skin tissue and reduction of scaly lesions which are deficient in essential fatty acids (Aburjai and Natsheh, 2003).

3.3. Biodiesel characteristics of the five selected foliage oils

Investigations have now been carried out on the fuel properties of different vegetable oils. Therefore, the current work is focused on assessing the vegetable oils' suitability to biodiesel. The five chosen vegetable oils were described in terms of thermo-physical/biodiesel properties through empirical connections.

3.3.1. Thermo-physical properties

Depending on the properties of esters present in the vegetable oils, the thermo-physical properties are considered one of the deciding properties of biodiesel (Montero and Stoytcheva, 2011). The theoretical evaluation of five vegetable oils' thermo-physical properties was carried out.

3.3.2. Density

Density is the critical parameter that directly influences many fuel properties of biodiesel such as cetane number, heating value, combustion and atomization characteristics. *R. vesicarius* showed the highest density (956.76 kg/m^3) among all the experimental samples**.** Other samples, such as *B. rubra*, *B. alba*, *H. sabdariffa*, and *H. cannabinus* have shown good density in the extracted oils in the range of 870 to 875 kg/m^3**,** which falls in the range of the biodiesel standards (860-900 kg/m^3). In general, an engine's output power depends on the density of the biodiesel. Hence it could be concluded that all the selected samples come close to the international standards for biodiesel. A study on the evaluation of waste and the efficiency of refined cooking oil to be used as biodiesel was also carried out. Waste cooking oil showed 916 kg/m^3 and refined cooking oil showed 913 kg/m^{3,} slightly lower than pure water at 998 kg/m^3 (Chuah *et al*., 2017).

The present data is comparable to several vegetable oils such as peanut, soybean, babassu, palm, and sunflower in the range of 860 to 883 kg/m^3 (Shereena and Thangaraj, 2009).

3.3.3. Kinematic viscosity

As shown in Table 2, the kinematic viscosity of the oils varied between 3.8-5.66 $\times 10^{-6}$ m^2/s. The highest kinematic viscosity was observed in *R. vesicarius* (5.66×10^{-6} m^2/s), which may be due to the presence of large molecular mass, followed by *B. rubra* (4.84×10^{-6} m^2/s), *B. alba* (4.84×10^{-6} m^2/s), *H. sabdariffa* (4.28×10^{-6} m^2/s), and *H. cannabinus* (3.81×10^{-6} m^2/s). Shereena and Thangaraj reported that palm oil showed the highest (5.7×10^{-6} m^2/s) and babassu oil showed the least (3.6×10^{-6} m^2/s) kinematic viscosity compared to other vegetable oils and commercial diesel (3.06×10^{-6} m^2/s) (Shereena and Thangaraj, 2009).

3.3.4. Higher heating value

The higher heating value is the determination of heat released when a one-unit volume of fuel is combusted. Among the experimental vegetable oils analyzed, the maximum heating value was observed in *R. vesicarius* (43.65 MJ/kg) as compared to the requirement of biodiesel fuels (> 45 MJ/kg). *B. rubra* (39.14 MJ/kg) and *B. alba*, *H. cannabinus* showed similar values (39.14 MJ/kg). *H. sabdariffa* lower highest heating value (37.72 MJ/kg) compared to other vegetable oils extracted. This variation in the highest heating values is due to the presence of chemically-bound oxygen atoms. These values are higher compared to the reported commercial vegetable oils where babassu oil showed a lower value (31.8 MJ/kg) than peanut and soybean (33.5 MJ/kg) (Shereena and Thangaraj, 2009). The heat of combustion of all the selected GLVs samples was recorded in the range of 54659 to 55239 kJ/kg.

3.3.5. Viscosity

Viscosity is one of the important physical properties which provides information about the resistance of the fluid in biodiesel. The qualities of biodiesel such as the size of the fuel drop, jet penetrations, and atomization depended on viscosity.

Table 2. Predicted biodiesel properties in the five selected GLV foliage oils.

Parameter	ASTM D6751 Standards	*H. cannabinus*	*H. sabdariffa*	*B. alba*	*B. rubra*	*R. vesicarius*
Density (kg/m^3)	860-900	871.31 ± 0.00	875.11 ± 0.00	870.96 ± 0.00	870.96 ± 0.00	956.76 ± 0.00
Kinematic viscosity (m^2/s) ($\times10^{-6}$)	1.9-6.0	3.81 ± 0.01	4.28 ± 0.01	4.84 ± 0.01	4.84 ± 0.01	5.66 ± 0.01
Higher heating value (MJ/kg)	*	39.36 ± 0.00	37.72 ± 0.03	39.14 ± 0.05	39.14 ± 0.05	43.65 ± 0.00
Viscosity (N.m^{-2}. s)	*	104.58 ± 14.62	103.64 ± 14.19	104.04 ± 14.27	104.34 ± 14.31	104.51 ± 14.50
Saponification Value (SV)	164-220	205.62 ± 0.02	202.18 ± 0.33	203.17 ± 0.06	204.29 ± 0.03	205.34 ± 0.01
Cetane Number (CN)	>47	34.67 ± 0.009	33.99 ± 0.12	44.05 ± 0.005	60.51 ± 0.003	34.75 ± 0.009
Iodine Value (g I_2/100 g)	<140	169.63 ± 0.03	174.67 ± 0.34	129.38 ± 0.05	55.55 ± 0.01	169.44 ± 0.03
Degree of unsaturation (DU)	*	136.57 ± 0.03	140.96 ± 0.26	117.64 ± 0.06	52.41 ± 0.01	138.66 ± 0.16
Long chain saturated factor (°C)	*	5.56 ± 0.03	3.32 ± 0.01	7.83 ± 0.01	14.34 ± 0.04	4.84 ± 0.01
Cold filter plugging point (°C)	*	-0.88 ± 0.10	-6.04 ± 0.01	8.13 ± 0.01	28.60 ± 0.12	-1.24 ± 0.05
Heat of combustion (kJ/kg)	*	54812.28 ± 0.73	54659.29 ± 11.20	54886.83 ± 2.58	55239.04 ± 1.22	54802.16 ± 0.86
Elemental composition (%)						
Carbon (C)	86.5	72.74 ± 1.30	66.38 ± 1.26	75.51 ± 0.17	76.86 ± 0.12	71.24 ± 1.50
Hydrogen (H)	13.5	14.20 ± 0.49	10.78 ± 1.08	13.89 ± 0.12	16.24 ± 0.09	15.60 ± 0.29
Nitrogen (N)	*	3.00 ± 0.21	3.13 ± 0.14	1.58 ± 0.03	0.71 ± 0.01	2.79 ± 0.41
Sulphur (S)	*	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.08 ± 0.00	0.00 ± 0.00
Carbon/Hydrogen (C/H)	6.24	5.12 ± 0.08	6.15 ± 0.51	5.43 ± 0.04	4.73 ± 0.02	4.56 ± 0.06

All values represented are mean ± SD of three replicates analyzed. * American Society for Testing and Materials (ASTM) standard values were not available for these parameters. Biodiesel values are based on standard conversion factor and empirical formulas. CHNS (Carbon, Hydrogen, Nitrogen, Sulphur) values.

Fuel viscosity has both upper and lower limitations (3.5-5.0 $N \cdot m^{-2} \cdot s$). Low viscosity causes leakage problems and higher viscosity causes poor fuel atomization. The experimental vegetable oils contained almost the same amount in all the samples, in the range of 103.6-104.5 $N \cdot m^{-2}.s$.

3.3.6. Cetane number (CN)

CN is a key indicator of fuel quality, and it characterizes the fuel's ease of combustion. The higher cetane values indicate the smooth running of the engine, and according to the ASTM standards, it should be greater than 47 (Montero and Stoytcheva, 2011). In the present study, a higher CN 60.51 was observed in *B. rubra* followed by *B. alba* (44.05), *H. sabdariffa* (34.99), *R. vesicarius* (34.75), and *H. cannabinus* (34.67) (Table 2). Compared to the standard CN 28% more was observed in *B. rubra,* and the other four showed lower CN values than the standard. The CN of *B. rubra* oil (60.51) is comparable to the CN of palm oil (62). Similarly, the commercial diesel was recorded to show a CN of 50 as reported by (Shereena and Thangaraj, 2009).

3.3.7. Iodine value

Iodine value is the standard marker for biodiesel quality, which reveals the biodiesel's stability to oxidation. It is estimated by the nearness of unsaturated fatty acids and ester composition. Biodiesel with a higher iodine value provides ease in oxidation when in contact with air. Among the experimental samples, the most elevated was seen in *H. sabdariffa* (174.67 g I_2/100 g). *H. cannabinus* and *R. vesicarius* presented practically comparable iodine levels (169 g I_2/100 g). The lowest iodine value was seen in *B. rubra* (55 g I_2/100 g). Biodiesel with a higher iodine value causes polymerization and deposits on piston rings and injector nozzles. The standard iodine value detailed in different nations indicates that in Japan and Europe it is 120, and in South Africa 140; while in India and Australia iodine values were not considered to assess the biodiesel nature of the fuel.

3.3.8. Biodiesel cold-flow properties

At cold temperatures, the performance of the oil/biodiesel will vary and worsen fuel flow. It is important to characterize the biodiesel's cold properties. Mostly, these properties depend on the fatty acid composition of the triacylglycerol. Higher molecular weight triacylglycerol is responsible for poor cold-flow characteristics of biodiesel.

3.3.9. Cold-filter plugging point (CFPP)

CFPP is the indicator of flow performance of biodiesel at lower temperatures. The CFPP of vegetable oils produced from various feedstock is shown in Table 2. The results showed that the highest was observed in *B. rubra* (28.60 °C), which is due to a higher long-chain saturated factor of 14.34 °C, indicating the presence of higher long-chain fatty acids. *B. alba* (8.13 °C), *H. cannabinus* (-0.88 °C), *R. vesicarius* (-1.24 °C), and *H. sabdariffa* (-6.04 °C**)** were observed due to present lower LCSF values at 7.83, 5.56, 4.84, 3.32, respectively, indicating the presence of lower long-chain fatty acids. The presence of long-chain fatty acids decreases the properties of biodiesel at cold temperatures. Due to the presence of Arachidic acid and Lignoceric acid in *B. rubra*, the oil showed higher CFPP values. CFPP values are climate dependent, and for temperate climate conditions they are reported to be in the range of -20 to 5 °C, except for *B. rubra* and *B. alba,* which are comparatively within the range for temperate conditions (Montero and Stoytcheva, 2011).

3.4. Carbon, Hydrogen, Nitrogen and Sulphur (CHNS) contents

Table 2 shows the elemental (CHNS) composition of oil extracted from selected GLV foliage. In the present study, the carbon and hydrogen components were seen as the most noteworthy in all the oil samples with 66 to 76% carbon and 10 to 16% hydrogen. In any case, there was less substance of nitrogen with 0.71 to 3%. There was an immaterial amount of sulfur in all the oil samples chosen. A comparable pattern in elemental composition was additionally detailed in *Pithecellobium dulce* seed oil with carbon as most noteworthy (76%) and no sulfur detected (Sekhar *et al*., 2018). It was determined that this synthesis is practically identical and that commercial diesel properties include 85% carbon and 0.15% sulfur. A comparative pattern of bio-diesel attributes was recorded for all the chosen GLVs.

4. CONCLUSIONS

The information on total oil and fatty acid profiles uncovered the distinctions among the chosen GLV fo-

liage. Despite the fact that the significant fatty acids distinguished were the same in four foliage powders aside from *B. rubra*, which was affirmed by MS. The major fatty acids of the selected foliage showed that in *Hibiscus* spp. C18:3 (49.3 µmol % and 50.4 µmol %) were recorded to be the highest, followed by C16:0 (23.2 µmol % and 21 µmol %) in *H. cannabinus* and *H. sabdariffa,* respectively. However, *B. rubra* showed the highest TUSFA at above 65% with the significant composition of PUFA above 52%. The inferences from the bio-diesel properties showed that aside from *R. vesicarius,* every single vegetable oil was within the scope of bio-diesel measurements. Kinematic viscosity and saponification values for all the oils were inside the range of (3.81 to 5.66×10^{-6} m^2/s and 202 to 206 $N.m^{-2}.s$), which lie within the ASTM standard range (164-220 $N.m^{-2}.s$). However, as for cetane number (60.51), *B. rubra* indicated values in the scope of bio-diesel properties (> 47). In cold flow properties, for example, LCSF and CFPP demonstrated that *H. cannabinus, H. sabdariffa* and *R. vesicarius* could be utilized in temperate conditions. It is important to carry out a comparative study on the fatty acid profile and bio-diesel qualities of the chosen foliage which could be helpful for these oils as vegetable oil for human consumption and in bio-diesel applications.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Biotechnology, Government of India, New Delhi, for financial assistance (BT/PR1238/FNS/20/524/2011). We greatly acknowledge the Director, CSIR-CFTRI for their kind support.

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