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Original Research Article

Synthesis of biodiesel from *Reseda luteola* L. seeds and its chemical characterization using GC and GC-MS instrumentations

Ali Shafaghat[1, ✉], Parisa Morabbi[2], Masoud Shafaghatlonbar[3] and Farshid Salimi[2]

[1]Department of Chemistry, Khalkhal Branch, Islamic Azad University, Khalkhal, Iran
[2]Department of Chemistry, Ardabil Branch, Islamic Azad University, Ardabil, Iran
[3]Department of Chemistry, College of Sciences, University of Birjand, Birjand, Iran

ABSTRACT

The present study reports the general procedure for the preparation of biodiesel from *Reseda luteola* L. seeds oil using normal hexane as an organic solvent. The hexane extract was obtained by Soxhlet apparatus and subjected to transesterification method to prepare simple esters. The chemical composition of the prepared biodiesel was determined by using GC and GC/MS instruments. Thirty-two components representing 89.8% of the extracted oil of *R. luteola* were identified, among them, 9,12-octadecadienoic acid methyl ester (23.8%), 9-octadecenoic acid methyl ester (12.2%), hexadecanoic acid methyl ester (9.9%) and *n*-decane (6.1%) were the prevailing compounds. The organic extract of seeds from *R. luteola* detected as an important source of unsaturated fatty acid ester compounds.

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1. Introduction

Biodiesel is obtained from different herbal oils, animal fats or used cooking oils and can be used in the diesel motors (Adekunle et al., 2016; Sales et al., 2017; Sena and Pereira, 2017; Silveira et al., 2017; Solis et al., 2017; Yang et al., 2017). It has already been established as an alternative, non-toxic, biodegradable and renewable diesel fuel (Goodrum and Geller, 2005; Usta, 2005; Leung et al., 2010; Yaliwal et al., 2016; Mahmudul et al., 2017; Pelegrini et al., 2017; Singh et al., 2017; Sundus et al., 2017). Biodiesel is defined as the mono alkyl esters of long chain fatty acids and is typically produced through transesterification of oils and fats with methanol or ethanol in the presence of different acidic and alkaline catalysts (Foidl et al., 1996; De and Bhattacharyya, 1999; Ma and Hanna, 1999; Fernandes and Ferreira-Dias, 2001; Zullaikah et al., 2005).

Plant seeds and kernels are always considered as important sources of proteins and essential fatty acids and are widely used in nutritional and pharmaceutical industries (Eromosele, 1997). In literature, seeds and kernels of numerous species of the plants which are rich sources of fatty acids are of great importance as herbs and spices. During the recent years, chemical compositions of the essential oils and/or organic extracts of the plant materials have come more into the focus of phytochemistry and green chemistry (Singh et al., 2005; Malti et al., 2008; Amiran et al., 2015). Their widespread use has raised the interest of scientists in basic research of fatty acids and for the preparation of biodiesel from natural materials. Fatty acid compounds can be separated from herbal materials by water or organic extraction, vapour distillation, or precipitation with lead or other elements (Joslyn, 1970; Knothe, 2008; Singh and Singh, 2010). Today, the most common way used for the extraction of fatty acids present in seeds, fruits and vegetables is the solvent extraction (Goodrum and Geller, 2005; Park et al., 2008).

The genus *Reseda* is represented in Iranian flora by ten species, among which *R. macrobotrys* and *R. bungei* are endemic (Mozaffarian, 2007). The

✉ Corresponding author: Ali Shafaghat
Tel: +98-452-4251220-22, Fax: +98-451-7742659
E-mail address: shafaghata@yahoo.com

extracts of *R. luteola* display antiproliferative and pro-apoptotic properties (Woelfle et al., 2010) as well as antioxidative, antiinflammatory and photoprotective activities *in vitro*, *ex vivo* and *in vivo* (Wolfle et al., 2010). In addition, extraction of luteolin extracts (Cerrato et al., 2002), HPLC-based identifications of flavonoids from *R. luteola* (Cristea et al., 2003; Moiteiro et al., 2008) have been subjects of some reports. Recently, neuroprotective effect from an aqueous extract of *R. luteola* has been documented in the literature, as well (Kim et al., 2015).

In continuation of phytochemical studies on the medicinal plants from North-West of Iran, in particular *Reseda* species, the Iranian *R. luteola* (Resedacea) was investigated. To the best of our knowledge, no phytochemical studies on *R. luteola* have been reported, but our primary study showed the presence of tannin, saponin and flavonoid in this species. The main active constituents of these plants are also responsible for their colour and therapeutic efficacy. This article is the first report on the isolation and preparation of biodiesel by using transesterification method on the fatty acid from seeds of *R. luteola* from Iran.

2. Experimental

2.1. Plant material

The seed parts of *Reseda luteola* (Fig. 1) were collected from Khalkhal area, Ardabil province (Fig. 2) in August 2016 in northwest of Iran at an altitude of 1550 m. A voucher specimen (No: 027) has been deposited at the Herbarium of the Agriculture Research Centre (A.R.C.) Ardabil, Iran.

Fig. 1. The photograph of *Reseda luteola* L.

2.2. Extraction of *n*-hexane sample extract

Dried and powdered material (seeds) was extracted with *n*-hexane using a Soxhlet apparatus (70 ºC, 2.5 h) to obtain the non-polar components. During the extraction procedure, analytical reagent grade *n*-hexane (98%) was used as an extracting solvent. The obtained extracts were concentrated by rotary evaporator under vacuum at 45 ºC.

2.3. Methylation of hexane extract

After removing hexane using rotary evaporator, the oily mixtures were derived to their methyl esters according to the general guidelines given by the International Olive Oil Council (IOOC) (http://www.internationaloliveoil.org) and IUPAC (Ulberth and Haider, 1992) reports by the transesterification process. In this process, dried *n*-hexane extracts were again dissolved in hexane and then extracted with a methanolic solution of KOH (2.0 M) at room temperature for 2 min. The upper phase was subsequently analyzed by means of GC/FID and GC/MS systems.

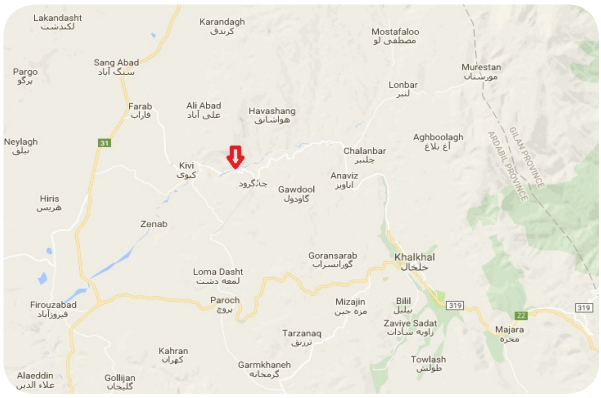

Fig. 2. The geographical map of the plant (*Reseda luteola*) in the sampling area.

2.4. GC analysis

GC analysis was performed on an Agilent 7890A gas chromatograph equipped with a split/splitless injector (250 ºC) and a flame ionization detector (250 ºC). Helium was used as carrier gas (1 mL/min) and the capillary column used was DB-5 (30m × 0.2 mm, film thickness 0.32 µm). The column temperature was kept at 40 ºC for 5 min and then heated to 230 ºC with a 6 ºC/min rate and kept constant at 230 ºC for 5 min. The relative percentages of the characterized components are given in Table 1.

2.5. GC/MS analysis

GC/MS analysis was performed using an Agilent 5975 with an HP-5MS column (30 m × 0.25 mm, film thickness 0.32 µm). The column temperature was kept at 40 ºC for 5 min and programmed to 230 ºC at a rate of 6 ºC/min and finally kept constant at 230 ºC for 5 min. The flow-rate of helium as carrier gas was 1 mL/min. MS were taken at 70 eV. The fatty acids were identified through comparing their retention times and mass spectral peaks with those of standard methyl ester mixtures and by an overall search in the NIST-Wiley library data. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A chromatopac without the use of correction

Table 1
Chemical compositions of fatty acid esters in seed of *R. luteola*.

NO.	Compound	Rt*(min)	%
1	*n*-Octane	3.31	0.5
2	1,2-Dimethyl-cyclohexane	3.86	0.5
3	Nonane	5.29	3.4
4	Propyl cyclohexane	6.07	1.1
5	2,6-Dimethyl octane	6.14	2.7
6	3-Ethyl-2-methyl heptane	6.34	2.1
7	1,1,2,3- Tetra methyl cyclohexane	6.77	0.7
8	4-Methyl-nonane	6.89	2.1
9	2-Methyl nonane	6.96	1.3
10	3-Methyl-nonane	7.15	1.4
11	1-Methyl-2-propyl cyclohexane	7.50	0.6
12	***n*-Decane**	**7.97**	**6.1**
13	4-Methyl decane	8.63	0.7
14	1,3-Dichloro-benzene	8.99	0.5
15	Undecane	10.91	0.8
16	Dodecane	13.83	2.8
17	5-Methoxy-1,2,3,4,5-pentamethyl-1,3-cyclopentadiene	13.96	0.5
18	Decahydro-2,3-dimethyl naphthalene	14.14	0.7
19	Decahydro-1,5-dimethyl naphthalene	14.69	2.4
20	Unknown	16.86	2.9
21	Tetradecane	19.26	1.2
22	Hexadecane	24.11	1.2
23	Alloaromadendrene	25.93	2.2
24	Octadecane	28.48	0.8
25	(+)-γ-Costol	29.73	1.6
26	**Hexadecanoic acid, methyl ester**	**31.05**	**9.9**
27	Dodecanoic acid methyl ester	31.48	1.0
28	Hexamethyl benzene	31.83	2.1
29	*n*-Eicosane	32.45	0.5
30	**9,12-Octadecadienoic acid, methyl ester**	**34.26**	**23.8**
31	**9-Octadecenoic acid, methyl ester**	**34.36**	**12.2**
32	10-Octadecenoic acid, methyl ester	34.44	1.1
33	Octadecanoic acid, methyl ester	34.80	1.3
	Total	**--**	**92.7%**

*Rt: Retention time

factors.

3. Results and Discussion

3.1. Chemical composition of the oil from the seeds of *R. luteola*

The oil was obtained from *R. luteola* seeds through the Soxhlet extraction method utilizing *n*-hexane as solvent. The yield of the oil was found to be 28% in terms of seeds weight. The results obtained in the analyses of the oil are listed in Table 1, in which the percentage and retention indices of constituents are given. According to the results (see Fig. 3), the major saturated and monounsaturated components including 9,12-octadecadienoic acid methyl ester, 9-octadecenoic acid methyl ester, hexadecanoic acid methyl ester and *n*-decane were characterized in the oil profile from the seeds of *R. luteola*.

As shown in Table 1, The major unsaturated fatty acids (UFA) were 9,12-octadecadienoic acid methyl ester and 9-octadecenoic acid methyl ester. Furthermore, about 89.8% (32 compounds) of the organic extract obtained from seeds were characterized. The main components in the *n*-hexane extract from the seeds of *R. luteola* were 9,12-octadecadienoic acid methyl ester (23.8%), 9-octadecenoic acid methyl ester (12.2%), hexadecanoic acid methyl ester (9.9%) and *n*-decane (6.1%). The unsaturated fatty acid esters contents (36.1%) were higher than saturated ones (9.9%). Hexadecanoic acid methyl ester as the main saturated fatty acid ester was presented in the seed oil sample. One of the principal fatty acid ester (9,12-octadecadienoic acid methyl ester) was a predominant component in seed of *R. luteola*. Unsaturated fatty acids ester have also been reported to be as effective as polyunsaturated fatty acids (PUFA) to lower low density lipoprotein cholesterol in humans (Mensink and Katan, 1989; Kanya et al., 2007; Wheat and Currie, 2007; Tan et al., 2009).

3.2. Biodiesel characteristics

Biodiesel was obtained from *R. luteola* seeds oil by using the transesterification route with potassium hydroxide as catalyst at room temperature. The fatty acids can initiate engine corrosion and affect human and animal health by dangerous transpirations. Therefore, the maximum allowable amounts of free fatty acids are included in the biodiesel determination of most countries (http://www.cyberlipid.org/glycer/biodiesel.htm). Potassium or sodium ions (K^+/Na^+) as ''alkali metals'' are used as catalysts in the preparation of biodiesel and should be removed through the biodiesel production process. This is mainly because the residual alkali metals can form deposits in fuel injection system. The constituents of biodiesel, including the fatty acid ester content and minor composition depend on the feedstock (Moser, 2008), which in turn can considerably influence the corresponding chemical and physical properties. Thus, the better execution of the binary mixtures could be generally explained by the suitable change in the constituents of the relating binary mixtures with respect to that of the original two biodiesels (James et al., 2011).

It has been shown that mixing of the biodiesels produced from different fatty acid ester sources, causes a remarkable change in the chemical and physical properties of the mixture and can also be affected by mixing biodiesels obtained from a single feedstock by transesterification with different alcohols (except MeOH) (Dunn, 2009).

4. Concluding remarks

In conclusion, the low cost and availability of fats and oils sources are among the main points in the production of biodiesel. By collecting utilized frying oils and their conversion to biodiesel, the price of biodiesel is remarkably lowered and the negative impact of disposing of the oil to the environment reduced. The prepared biodiesels from this seeds

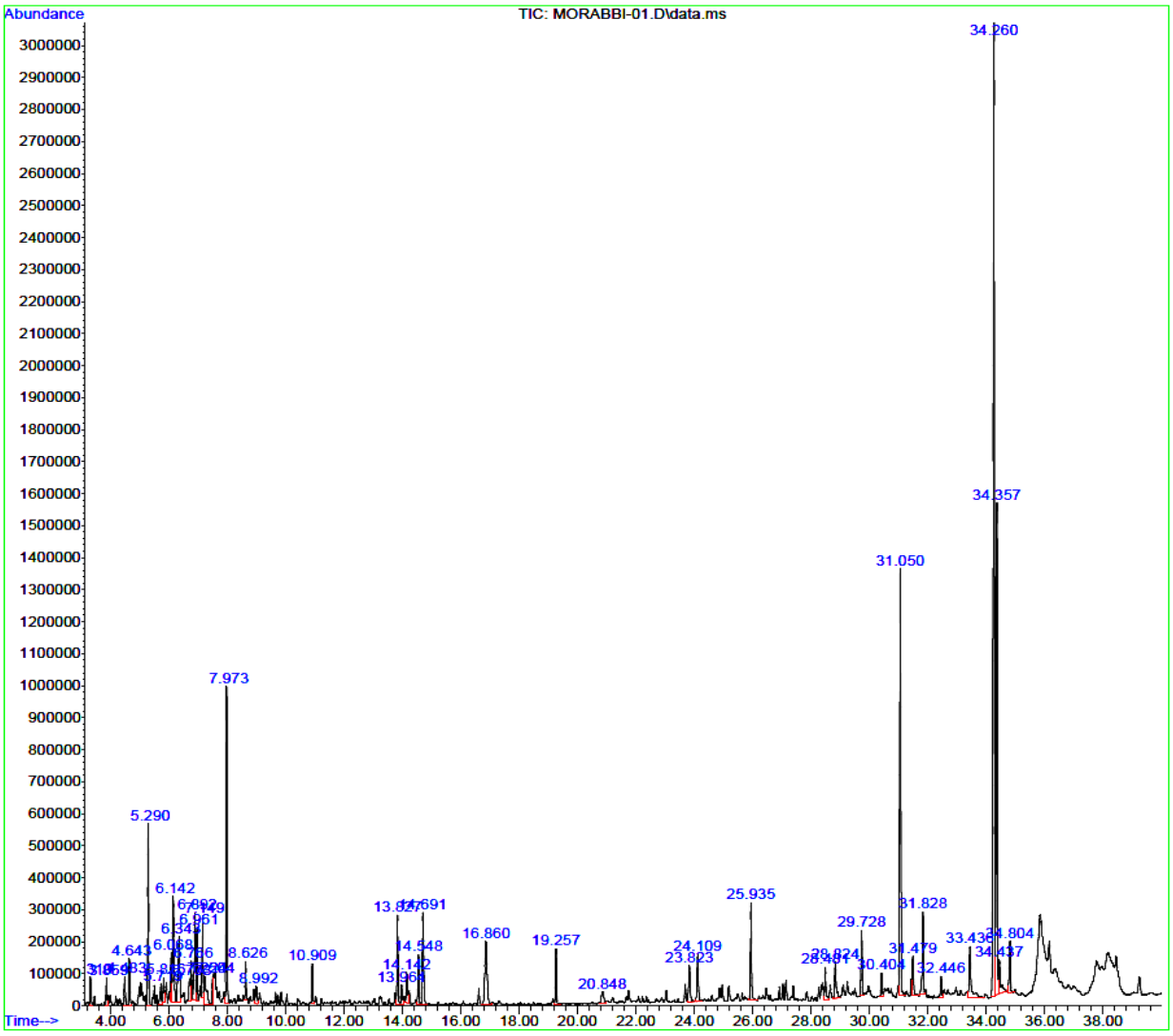

Fig. 3. The chromatogram from the seed oil (biodiesel) of *R. luteola*.

of *R. luteola* possess significantly low temperature properties. Further studies should be planned in order to: i) characterize the chemical, physical and mechanical properties of this biodiesel in detail and ii) elucidate the impact of geographical location and soil composition on the aforementioned properties. Results in the present study could be an effective introduction to the provided evidence that hexane extract of *R. luteola* seeds may provide a potential natural biodiesel for chemical and petrochemical industries. However, further studies are urgently needed to screen the chemical and physical properties. The presence of the components of *R. luteola* herb shown in Table 1 suggests the presence of fatty acid methyl ester compounds which have biodiesel properties.

Acknowledgments

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