Supplementary Materials: Optimization, Transesterification and Analytical Study of *Rhus typhina* Non-Edible Seed Oil as Biodiesel Production

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S1. Description of *Rhus typhina* (Sumac fruit)

We generated biodiesel from the *Rhus typhina* seed oil and studied their potential for biodiesel production. *Rhus typhina* is a member of family Anacardiaceae, also called sumac staghorn. It is native to American that can reach a height of 30–35 feet deciduous shrub to a small tree. It has 16 to 24 inches long alternating composite leaves, which are narrows or round at the base, and the tips sharply have serrated edges. The leaflet top is smooth and is dark green, and the bottom is pale except for the midrib. From June to July, a cluster of compact yellow-green flowers blooms. From August to September, the fruit reaches the mature level. The fruit is called stone fruits, which contains in a group of compact round red and hairy form. The diameter of each stone fruit is 6.35mm, and fruit exists in it. The drupes of each set may contain around 700 to 812 seeds.

The seeds contained 20–22% oil by (Soxhlet extraction) and 13.3% by (mechanical extraction). The oil can be easily converted to biodiesel using a base-catalyzed transesterification process. The fruit is produced on 3 to 4 years old plants. Mostly *Rhus typhina* tree has different populations of male and female plants, and only female plants can produce seeds. Male and female flower plants are occasionally found. The Sumac fruit seeds germination is heightened by their digestion through the rabbit, ringneck, and ticks. The fire presence also helps and contributes to the germination process increasing. Approximately one pound contains 60,000 seeds. For wildlife, Sumac fruit is generally used as a winter substitute diet. Different kind of birds includes sumac fruit as a diet such as white pelicans, ring-necked pheasants, wild turkeys, and almost 300 other species, also considered to be important dieting for Sharp-tailed Grouse and matsutake Grouse. Painted bark becomes a diet for fox squirrels and cottontail rabbits. Fruits and stems become a diet of white-tailed deer. It is best suited for severely disturbed locations that require pioneer species.

Fruits and stems become a diet of white-tailed deer. It is best suited for severely disturbed locations that require pioneer species. Sumac staghorn (*Rhus typhina* L.) is a perennial and flowering shrub belonging to the genus Rhus. Originated from Canada and the United States extensively dispersed in the northern hemisphere subtropical to temperate regions [1]. Its fruit has traditionally been used to make a drink called "Sumac-ade," which has been used as for a century a drink by the local people as a traditional medicine [2], which has a strong antibacterial and antioxidant activity [3,4]. Extensively cultivated in the part of North China since 1959 and is primarily used for horticulture and afforestation [5]. Mostly grow on the waste and barren land, which has no other purposeful use for edible crops.

S2.1. Material and reagents

From October to November 2018, at that stage had started seed collection from the wild plant when the seed color changed from green to brown. The immature and unhealthy seeds were separated while the healthy seeds (using heated deionized water) were washed to eliminate the soil. The oil was extracted using soxhlet and mechanical oil extractor (Table S1 Supporting Information). In the mechanical extraction process, the RT seeds were passed through two different electric oil extraction machines, low power extractor (Fangtai Shibayoufang FL-S2017 China), and high power

extractor (Fangtai Shibayoufang J508, China) and the oily coarse smear were collected in a beaker and used for further processing. Seeds pre-treatment is very important for soxhlet and mechanical extraction. Pre-treatment of the seed before the application of the mechanical extractor increases oil recovery. After 2–3 revolutions, a large amount of RT crude oil (RTSO) was obtained. Methanol 99–100% (Sinopharm Chemical Reagent Co., Ltd.), cyclohexane (Macklin) ≥ 99.9%, chloroform-d (Fluorochem), acetonitrile (Sigma Aldrich), petroleum ether (Sigma Aldrich), sodium hydroxide pellets ≥ 98% (Aladdin Shanghai, China), ethyl acetate 99.8% (Sigma Aldrich), dichloromethane 99% (J&K Scientific Ltd), potassium hydroxide pellets 99.99% (Aladdin), phenolphthalein pH 8.2–9.8 (Sigma Aldrich), starch, acetone (99%), sulphuric acid 98% (Aladdin), isopropanol, iodine and sodium iodide, purchased from Merck, Germany. Though all analytical reagent grade chemicals were used.

S2.2. GC-MS Procedure

The obtained RT biodiesel results were checked and tested by GCMS (QP2010SE, Shimadzu, Japan) furnished with a C. column: PEG-20M (30 m × 0.32 mm × 1 μm film thickness). Helium gas flow rate 1.2 ml/min; split ratio 40:1; the injector temperature and injection volume were 220 °C and 1 uL; Furnace heat up mode was 100 °C for 1 min, then from 100 °C rises to 210 °C at the increase rate of 10 °C/ min; sensor heat mode was 210 °C, then for 20 mins the temperature was continuing at 210 °C; ion source temperature 200 °C; for electron impact 70 eV ionization mode used; mass range 35–500 m/z. The Rhus typhina FAMEs were identified with the mass spectrometry fragmentation design provided by the GCMS system software, as matched with those stored in the mass spectrometry library No. NIST14 and their fatty acid identity were further verifying by matching with known standards values.

S3. ICP-OES and EA study of RT biodiesel for elemental analysis

For the presence of metals in the RTSO, biodiesel was explored through employing Inductively Coupled Plasma Spectrometer (Spectro-blue, Germany) and Elemental Analyzer (Vario EL CUBE, Germany). Both instruments ICP-OES (Spectro-blue, Germany) and EA (Vario EL CUBE, Germany) were used for specific elements analysis. ICP-OES Conditions: we first put a 1 g BD sample in a beaker and put it in the oven. The initial temperature was 0–200 °C for 1 h, then increase temperature to 500 °C for 2 h and finally 800 °C for 5 h. After completion was closed, the oven/ furnace and take out sample/ash and was dissolved in 2% HNO_3 (Nitric acid) to 10ml and was used that sample for elements finding and elements concentration of *Rhus typhina* biodiesel (RTBD).

Procedure for EA sample preparation: EA (Vario EL CUBE, Germany), Element analyzer instrument used for H, N, C, and O concentration RT biodiesel. Conditions we take 0.5 ml RTBD, 3 ml concentrated HCl (Hydrochloric acid), and 1ml Nitric acid in a tube and was put them for 10–15 mins rest, that oil can dissolve in solution. Would be used fresh reagents, the aqua regia amount would be double then sample. Then we take 1 ml that solution in a new bottle, and we add in them deionized water up to 5 ml. We repeated the same technique 2–3 times until the sample looks clean and clear, and we use that sample for testing C, H, N, and O.
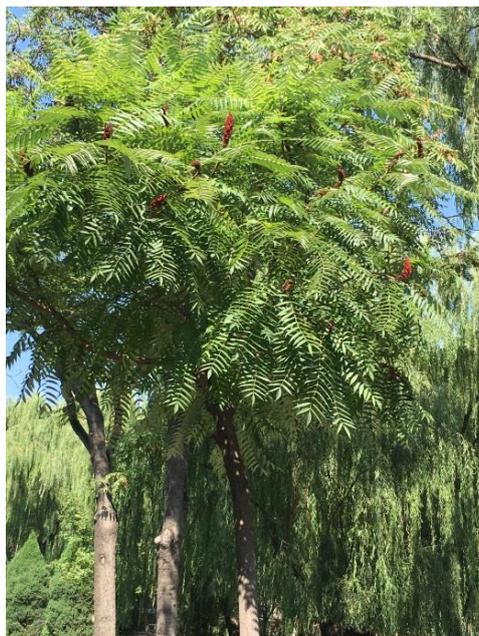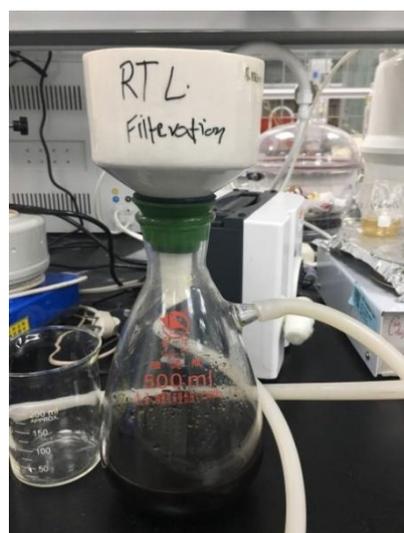
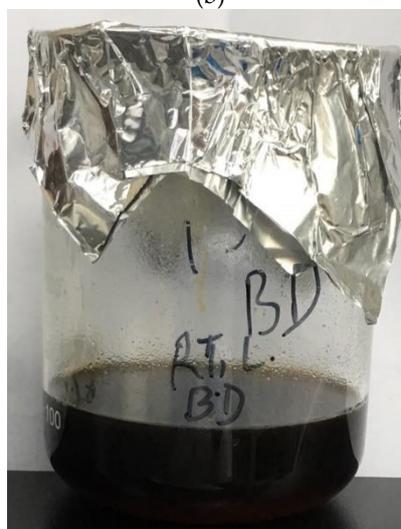**Figure S1.** *R. typhina plant* with fruit.
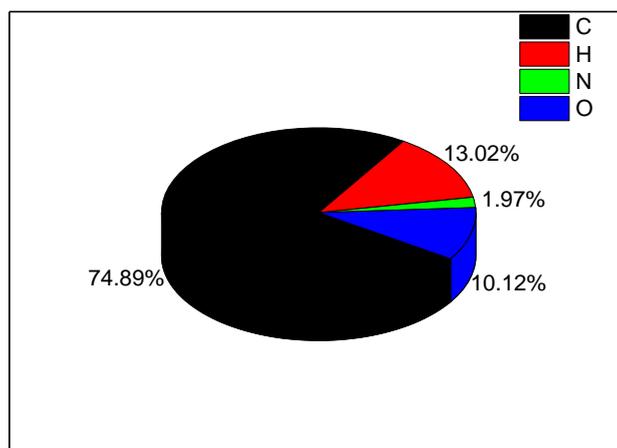

(a)

(b)

(c)

(d)

Figure S2. (a) RT crude oil filtration (b) optimization (transesterification) (c) BD washing (d) RT biodiesel.

Figure S3. RTBD EA (Elemental analysis) for C, H, O, and N.

Table S1. Source collection, oil extraction and transesterification of non-edible *R. typhina* (Sumac fruit) seed oil as biofuel.

Source name	Solvent extraction %	Mechanical extraction %	Biodiesel conversion %	Glycerin %	Soap %	Source collected from
Rhus typhina, L. (RT)	20–22	13.3	93.4	6.6	0	Binhai new area, Tianjin

Table S2. RT FAMEs detail process of optimization.

Amount of oil used (M/L)	Molar ratio of oil to alcohol	Temperature (°C)	Stirring intensity (rpm)	Reaction time (mint)	Amount of catalyst used		Percentage yield of various product		
					KOH (wt.%)	CH₃OH (ml)	Biodiesel (%)	Glycerol (%)	Soap (%)
12	**4:1**	**65**	**700**	60	4.7	3	75.16	16.5	8.30
12	5:1	65	700	60	3.6	2.4	81.7	13.3	5
12	**6:1**	**65**	**700**	**60**	**2.9**	**2**	**91.66**	**7**	**1.34**
12	7:1	65	700	60	2.0	1.7	70	20	10
12	6:1	65	700	60	2.0	2	83	12.5	4.16
12	6:1	65	700	60	2.4	2	88	10.3	1.7
12	**6:1**	**65**	**700**	**60**	**2.9**	**2**	**92**	**6.5**	**1.5**
12	6:1	65	700	60	3.8	2	87	8	5
12	6:1	60	700	60	2.9	2	82.5	13.3	4.16
12	**6:1**	**65**	**700**	**60**	**2.9**	**2**	**91.6**	**8.3**	**0**
12	6:1	70	700	60	2.9	2	88	9.16	2.5
12	6:1	65	500	60	2.9	2	78	21.6	0
12	6:1	65	600	60	2.9	2	90	10	0
12	**6:1**	**65**	**700**	**60**	**2.9**	**2**	**92.5**	**7.5**	**0**
12	**6:1**	**65**	**800**	**60**	**2.9**	**2**	**92.5**	**7.5**	**0**
12	6:1	65	700	40	2.9	2	75	20.8	4.16
12	6:1	65	700	60	2.9	2	90.8	9.16	0
12	**6:1**	**65**	**700**	**80**	**2.9**	**2**	**93.4**	**4.6**	**2**
12	6:1	65	700	100	2.9	2	88	10	2

Table S3. FTIR data presenting various functional groups in RT FAMEs.

Peak no.	Wavenumber (cm^{-1})	Group attribution	Vibration type	Abs intensity
1	3464	-OH	Stretching	Weak
2	3007	=C-H	Stretching	Strong
3	2925	-CH$_2$	Asymmetric stretching vibration	Strong
4	2854	-CH$_2$	Asymmetric stretching vibration	Strong
5	1743	-C=O	Stretching	Strong
6	1641	-CH$_2$	Shear type vibration	Middling
7	1435	Terminal Methyl	Stretching vibration	Higher
8	1361	-CH$_3$	Bending vibration	Middling
9	1170	C-O-C	Symmetric stretching vibration,	Middling
10	1016	C-O-C	Vibration	Weak
11	723	-CH$_2$	Plane rocking vibration	Weak

Table S4. ^{1}H NMR spectroscopic data showing the chemical composition of various methyl esters (Methoxy proton) in RT, biodiesel (FAMEs) sample.

Peak no	Peak area/ region/ ppm	Identified compound	Chemical structure
1	0.89	Terminal methyl protons	-CH$_3$
2	0.99	Terminal methyl Protons	-CH$_3$
3	1.27	β-methyl protons	-CH$_2$
4	1.31	β-methyl protons	-CH$_2$
5	1.62	β-methyl protons	-CH$_2$
6	2.04	α −methylene protons	-CH$_2$
7	2.30	α-methylene proton	-CH$_2$
8	2.805	α-methylene protons	-CH$_2$
9	3.66	Methoxy proton	-OCH$_3$
10	5.34	Olefinic protons	-HC=CH

Table S5. ^{13}C NMR spectroscopic data showing the chemical shift values corresponding to various structural features in RT (Methoxy carbon) FAMEs.

Peak no:	Peak area/ region/ ppm	Identified compound	Chemical structure
1	14.1	Terminal methyl carbon	-CH$_3$
2	22.57	Methylene carbon	-CH$_2$
3	34.1	Methylene carbon	-CH$_2$
4	51.32	Methoxy carbon	-OCH$_3$
5	127.89	Olefinic carbon	C=C
6	130.21	Olefinic carbon	C=C
7	174.24	Carbonyl carbon of ester	-COOCH$_3$

Table S6. Comparison ICP-OES elements concentration (ug/g) of RT-BD with petro-diesel.

Name of elements	Ele. Conc in μg/L	Ele.Conc in μg/g	Petro-diesel ug/g
Na	65.37	70.29	868.3
K	2.83	3.219	213.3
Al	48.84	55.56	-
Zn	43.86	49.89	9.5
Ni	41.3	46.98	12.4
Pb	35.03	39.85	-

Ca	6.47	7.360	21.4
Mg	28.78	32.74	35.6
Cu	27.97	31.82	99.6
Bi	25.54	29.05	-
V	13.56	15.42	-
Co	7.005	7.96	21.2
Mn	4.908	5.58	1.5
Cd	1.403	1.59	-
Tl	0	0	0

References

1   Rayne, S.; Mazza, G. Biological activities of extracts from Sumac (Rhus spp.). A review. *Plant Food Hum. Nutr.* **2007**, *62*, 165–175.
2   Foster, S.; James, A.D. A field guide to medicinal plants and herbs of eastern and central North America. Peterson, R.T., Ed.; Houghton Mifflin Harcourt: New York, NY, USA, 2000.
3   Kossah, R.; Zhang, H.; Chen, W. Antimicrobial and antioxidant activities of Chinese sumac (*Rhus typhina L.*) fruit extract. *Food Control* **2011**, *22*, 128–132.
4   McCune, L.; Johns, T. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest. *J. Ethnopharmacol.* **2002**, *82*, 197–205.
5   Wang, G.; Jiang, G.; Yu, S.; Li, Y.; Liu, H. Invasion possibility and potential effects of Rhus typhina on Beijing municipality. *J. Integr. Plant Biol.* **2008**, *50*, 522–530.