Non-edible plant seeds of *Acacia farnesiana* as a new and effective source for biofuel production

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Supporting Materials

S1. Plant description

In this study, *Acacia farnesiana* (AF) is chosen as the inedible raw material to produce biodiesel. Its scientific name is *Acacia farnesiana,* Family *Leguminosae (Mimosoideae*), native to North America. This tall semi-evergreen native shrub or small tree is commonly referred to as sweet acacia, Huisache, etc., with soft, medium-green feather-like, finely divided small leaves. The slightly thick stem is rich in chocolate brown or grey, with long and pointed needles. The small, puff-like yellow flowers are very fragrant, appear in clusters in late winter, and then occasionally spread out after each new flush, providing nearly four seasons of flowering. An area of about one hectare wills 91,500 kg of seeds yield, and the efficiency of oil per hectare is approximately 21,250 kg. The fruit is an elongated pod, 3 to 6 inches long, dry, and covered with hard skin, brown. Green colour attracts birds; squirrels and other mammals have no obvious littering problems and stick to the trees, which is very beautiful. The long-lasting fruit has a smooth appearance and contains seeds cherished by birds and other wildlife.

S2. **Mechanical extraction of** *Acacia farnesiana* **seed**

The mechanical extraction of AF seeds was done by two different electric oil expeller machines, FANGTAI SHIBAYOUFANG FL-S2017 China (less power extractor) and FANGTAI SHIBAYOUFANG J508, China (high power extractor). Pre-treatment of seed is essential for mechanical extraction, which can increase the amount of oil recovery. After 2-3 revolutions, a large yield of crude AFSO was obtained. Through mechanical extraction,8.7 wt.% oil content occurs. The oil removed from the seed by mechanical presses desires additional handling of extraction and filtration to produce a purer raw feedstock. The oil production of AFSO was calculated by the following equation (1).

$$\text{Conversion \%} = \frac{\text{Obtained seed oil weight}}{\text{Total seed weight}} \times 100 \qquad (1)$$

Further following steps were done to get the AFOB, filtration, rotary evaporation for access methanol, heating, trans-esterification, settling, separation, and washing.

S3. GC-MS Procedure

The obtained AF biodiesel results were checked and tested by GCMS (QP2010SE, Shimadzu, Japan), furnished with a capillary 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, and then for 20 min, the temperature was continuing at 210 ˚C; ion source temperature of 200 ˚C; for electron impact 70 eV ionization mode used; mass range of 35-500 m/z. The AF 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 NIST14, and their fatty acid identity was further verifying by matching with known standards values.

S4. ICP-OES procedure for AFOB elemental analysis

For the presence of metals in the AFSO biodiesel, it was explored through Inductively Coupled Plasma Spectrometer (Spectro-blue, Germany) and Elemental Analyzer (Vario EL CUBE, Germany). For the ICP-OES test, we take 1 g of oil sample for incinerating. The ashing process is as follows: Increase the oven temperature to 200 ˚C in one hour, then increase the heat to 500 ˚C and kept for 2 h, and finally increase to 800 ˚C and kept for 5 h. The ash was dissolved in 10 mL of 2 % HNO_3. The prepared sample was used for elements finding and concentration test of the KP biodiesel (AFBD).

S5. Elemental analyzer (EA) procedure for AFOB elemental analysis

Procedure for EA sample preparation: Element analyzer (Vario EL CUBE, Germany), the instrument was used todetecting the H, N, C, and O concentration of AF biodiesel. We take 0.5 mL of AFBD, 3 mL of concentrated HCl and 1 mL of Nitric acid in a tube and put them for 10-15 min rest, to dissolve the oil in the solution. Fresh reagents can be used for sample preparation. The aqua regia amount would be double than the sample. Then we take 1 mL of prepared solution in a new tube and add deionized water up to 5 mL. We repeated the same technique 2-3 times until the sample becomes clean and bright and use for C, H, N, and O concentration testing.

Table S1. Source collection, oil extraction and transesterification of non-edible AF seed oil as biodiesel

Source name	Soxhlet extraction	Mechanical extraction	Biodiesel conversion %	Glycerine %	Soap %	Source collection
Acacia Farnesiana	23	8.3	96	3	1	Collected from the wild field of Pakistan

Table S2. Catalysts effect on FAMEs conversion yield

Catalyst	Catalyst Conc. (wt%)	FAMEs %	Glycerine %	Soap %
KOH	2.0	81	14	5
	2.5	86	9	5
	3.0	94	5	1
	3.5	91	6.5	2.5
NaOH	2.5	83	13	4
	3.0	93.5	4.5	2
	3.5	93	5	2
CaO	2.5	87	9	4
	3.0	92	5	3
	3.5	91.5	7	1.5
CH_3ONa	2.5	88	8	4
	3.0	91.5	6.5	2
	3.5	90	7	3
CH_3OK	2.5	81	13	6
	3.0	91	7	2
	3.5	88	6	6
ZrO_2	2.5	85	10	5
	3.0	90	6	4
	3.5	88	7	5

Table S3. AF FAMEs detail process of optimization

Amount of oil used (M/L)	Molar ratio of oil to alcohol	Temp (°C)	Stirring intensity (rpm)	Reaction time (min)	Amount of catalyst used		Percentage yield of Different product		
					KOH (wt%)	CH_3OH (mL)	Biodiesel (%)	Glycerol (%)	Soap (%)
10	4:1	65	700	6	4.0	2.5	85	12	3
10	5:1	65	700	6	3.5	2	91	7	2
10	6:1	65	700	6	3.0	1.6	96	3	1
10	7:1	65	700	6	2.5	1.4	90	6	4
10	6:1	65	700	6	2.0	1.6	81	14	5
10	6:1	65	700	6	2.5	1.6	86	9	5
10	6:1	65	700	6	3.0	1.6	94	5	1
10	6:1	65	700	6	3.5	1.6	91	6.5	2
10	6:1	65	700	6	4.0	1.6	85	12	3
10	6:1	60	700	6	3.0	1.6	89	9	2
10	6:1	65	700	6	3.0	1.6	96	4	0
10	6:1	70	700	6	3.0	1.6	90	6	4
10	6:1	75	700	6	3.0	1.6	85	10	5
10	6:1	65	500	6	3.0	1.6	89	6	5
10	6:1	65	600	6	3.0	1.6	92	8	0
10	6:1	65	700	6	3.0	1.6	96	4	0
10	6:1	65	800	6	3.0	1.6	88	8	4
10	6:1	65	700	4	3.0	1.6	87	10	3
10	6:1	65	700	6	3.0	1.6	95	5	0
10	6:1	65	700	8	3.0	1.6	93	5	2
10	6:1	65	700	1	3.0	1.6	90	5	5

Table S4. FTIR data presenting various functional groups in AF FAMEs

Peak no.	Wavenumber (cm^{-1})	Group attribution	Vibration type	Absorption intensity
1	3006	=C-H	Stretching	Strong
2	2925	$-CH_2$	Asymmetric stretching vibration	Strong
3	2854	$-CH_2$	Symmetric stretching vibration	Strong
4	1743	-C=O	Stretching	Strong
5	1641	$-CH_2$	Shear type vibration	Middling
6	1361	$-CH_3$	Bending vibration	Middling
7	1170	C-O-C	Symmetric stretching vibration,	Middling
8	1016	C-O-C	Anti-stretching vibrations Vibration	Weak
9	723	$-CH_2$	Plane rocking vibration	Weak

Table S5. [1]H NMR spectroscopic data showing the chemical composition of various methyl esters (Methoxy proton) in AF biodiesel (FAMEs)

Integration value	Chemical shift ppm	Multiplicity	Inferences
3	0.88	Multiplet	CH_3 is attached to the aliphatic group.
16	1.27	Multiplet	A long aliphatic chain is present.
2	1.61	Quartet	CH_2 group is attachedto terminal CH_3.
3	2.03	Multiplet	CH_2 of long-chain aliphatic (Saturated) group.
2	2.29	Triplet	CH_2 group is attached with CH of long aliphatic (unsaturated/ Olefinic group).
1	2.77	Triplet	CH group is attached with an electron-withdrawing carbonyl group.
3	3.66	Singlet	Methoxy（OCH_3）group attached with an electron-withdrawing carbonyl group.
3	5.33	Multiplet	Olefinic hydrogen of a long-chain unsaturated aliphatic group

Table S6. ^{13}C NMR spectroscopic data showing the chemical shift values corresponding to various structural features in AF (Methoxy carbon) FAMEs

Peak no:	Peak area/ region/ ppm	Identified compound	Chemical structure
1	14.07ppm	Terminal methyl carbon	$-CH_3$
2	22.55-34.09ppm	Methylene carbon	$-CH_2$
3	51.37ppm	Methoxy carbon	$-OCH_3$
4	127.91-130.19ppm	Olefinic carbon	C=C
5	174.24ppm	Carboxyl carbon of ester	$-COOCH_3$

Table S7. Shows AF-BD ICP-OES detail elements concentration (µg/g) in comparison with petrodiesel

Name of elements	Elements conc. µg/L, µg/mL	Exp. Results Elements conc. µg/g	Petro-diesel elements conc.	The density of the Source Ac. F g/cm^3
Mg	**1.05**	**1.26**	**35.6**	0.83134
Zn	55.06	66.2	9.5	0.83134
Al	42.12	50.6	-	0.83134
Na	**21.0**	**25.2**	**868.3**	0.83134
Li	16.28	19.5	1.6	0.83134
Ni	9.586	11.5	12.4	0.83134
Ca	**8.58**	**10.3**	**21.4**	0.83134
S	1.02	**1.22**	**-**	0.83134
Mn	4.563	5.4	1.5	0.83134
V	4.422	5.3	-	0.83134
K	**4.08**	**4.9**	**213.3**	0.83134
Co	1.514	1.8	21.2	0.83134
P	**0.660**	**0.79**	**-**	0.83134

Table 8. AF FAMEs EA (elemental analysis) study for C, H, N, and O

Ultimate analysis	AF-BD	pistachio shell[1]	Peach Stones[2]	Apricot kernel shells[3]	Cherry Stones[4]	Mahua Seed[5]
C%	76.37	42.41	45.92	47.33	52.48	61.24
H%	13.34	5.64	6.09	6.37	7.58	8.40
N%	2.18	0.070	0.580	0.370	4.54	4.12
O%	8.11	51.87	47.38	45.93	35.30	25.50
Higher heating value (MJ/kg)	23.39	22.21	24.07	24.29	24.11	25.30

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