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Evaluation of *Aphanamixis polystachya* (Wall.) R. Parker as a potential source of biodiesel

K Rajesh Kumar, Channarayappa, K T Prasanna, Balakrishna Gowda*

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Abstract

Aphanamixis polystachya (Wall.) R. Parker (amoora), a promising oil yielding tree has been evaluated as a potential source for biodiesel. Amoora seeds contain 40-44 % oil with 63.4 % unsaturated fatty acids and 4.62 % Free Fatty Acids (FFA). A two stage process has been standardized and adopted for biodiesel production during the investigations. In acid pretreatment step, amoora oil was treated with 5 % H_2SO_4 based on FFA and 40:1 methanol to FFA by molar ratio in order to reduce FFA content. The second stage involved methanol and NaOH for alkali catalyzed transesterification. The maximum biodiesel yield was 96 % (v/v) with 1 h reaction time at 60 °C temperature and 1: 6 oil to methanol molar ratio. The viscosity of oil was reduced from 44.9 mm^2/s to 4.67 mm^2/s by transesterification. Biodiesel obtained was found to be on par with ASTM and BIS specifications.

Keywords: *Aphanamixis polystachya;* Biodiesel; FAME; Transesterification; Tree borne oils.

Introduction

The depleting reserves of fossil fuels and increasing demand for automobile fuels have triggered many initiatives in the field of alternative energy sources. The issue of biofuels as alternative fuels has gained momentum most likely because of its replenishable nature. Among many oil sources used for biodiesel production, the tree borne oils are gaining importance globally as a major alternative energy source for the existing petroleum fuels.

K Rajesh Kumar, K T Prasanna, Balakrishna Gowda*

Biofuel Park, Department of Forestry and Environmental Science, University of Agricultural Sciences, Bengaluru 560 065, India

Tel: +91-80-23620022, Fax: +91- 80-23330277
Email: gowdabk@yahoo.com

Channarayappa, K Rajesh Kumar

Department of Biotechnology, M. S. Ramaiah Institute of Technology, Bengaluru 560054, India

In India use of edible oils for biodiesel production is not recommended, since there is big gap between supply and demand (Anonymous 2008; Singh and Dipti 2010; Sarvesh et al. 2008). However, India has very diverse plant resources that produce non-edible oils and can be harnessed for biodiesel production. More than 400 oil yielding plant species across various agro-ecological regions of India have been reported (Anonymous 2008; Gowda et al. 2009). The annual estimate of tree borne oil seeds are more than 20 million tons (Ghadge and Raheman 2006). The cost of raw material accounts for more than 60 % of the total cost of biodiesel (Ma and Hanna 1999). A number of tree species that have significant oil content have been shortlisted with a few of them being found to possess more than 20 % of oil that is required to serve the purpose of biodiesel production (Chhetri et al. 2008; Kalayasiri et al. 1996; Mohibbe et al. 2005). Among these tree species, *Pongamia pinnata*, *Azadirachta indica*, *Madhuca indica*, *Simarouba glauca, Jatropha curcas* are well known for their high oil content and easier agronomic adaptations (Sarvesh et al. 2008; Gowda et al. 2009).

Aphanamixis polystachya (Wall.) R. Parker [Syn. *Amoora rohituka* (Roxb.) Wight &Arn.], commonly known as amoora, a member of the family Meliaceae, is native to tropical Asia. It is distributed in Indian subcontinent, Bhutan, Sri Lanka, Indo-China, Myanmar, Thailand, Malaysia, Indonesia, Papua, New Guinea and Philippines (http://www.flora.ac.cn). In South India, it is commonly found in the lower ghats and occasional in upper Western Ghats (Saldana and Nicolson 1976). This evergreen tree attains a height of 20-25 m. The tree starts yielding seeds at an age of 6 to 8 years with an average yield of 20 to 25 kg seeds/ tree. Average seed weight is approximately 0.7 to 0.9 g (Bhat 2003; Saldana and Nicolson 1976). Amoora tree has good medicinal value. The plant possesses antitumor, antimicrobial, hepatoprotective, insecticidal, depressant properties (Hossain et al. 2009). These trees are also planted as avenue trees. The rich seed oil content has made the tree a potential source for biodiesel.

Biodiesel is a mixture of mono-alkyl esters of fatty acids obtained through a chemical process called transesterification of oils and fats. In this process oils or fats are treated with an alcohol such as methanol or ethanol in the presence of acid, alkaline or enzymatic catalysts. In alkali catalyzed transesterification, sodium hydroxide (NaOH) or potassium hydroxide (KOH) is widely used. The

presence of free fatty acids (FFA) is crucial in determining the catalyst requirement for biodiesel production**.** The oils that do not contain FFA require 3.5 g of NaOH per liter of oil as catalyst. The excess FFA demands additional NaOH for neutralization which is determined by titration test **(**Dennis et al. 2010; Pelly and Michael 2009; Slinn and Kendall 2009**).** High FFA content leads to soap formation during the transesterification process. Further, formation of soap interferes with glycerol separation and washing of biodiesel. Therefore, the FFA content should be reduced below 3 % in the oil (Dennis et al. 2010; Ma and Hanna 1999; Sulistyo et al. 2009). The esterification process is carried out for high FFA oils to convert them into biodiesel using sulphuric acid catalyst and methanol. For the pretreatment process 5 % (w/w) of H_2SO_4 based on FFA and 40:1 methanol to FFA molar ratio is normally employed (Fan et al. 2009).

The present investigation mainly aims at qualitative and quantitative analysis of amoora oil; biodiesel production and evaluation of quality parameters of biodiesel.

Materials and Methods

Materials

The seeds were collected from trees planted as avenues around Bengaluru, India. The seeds were sun dried to attain constant weight (i.e., seeds with ≈7 % moisture).

Qualitative and quantitative analysis of amoora oil

Oil estimation

Dried seeds of amoora were finely ground and 3 g of dry powder was used for oil estimation. The oil was extracted with Soxtherm apparatus (Gerhardt, Germany) using petroleum ether (40 - 60 °C) as solvent. The residual petroleum ether and moisture was evaporated using hot air oven at 110 °C for one hour and desiccated. The oil content was estimated on weight basis and expressed as per cent oil.

Biochemical analysis of oil

Analysis of acid value, saponification value and iodine value were carried out as per standard methods (Jayaraman 1981). The density (hydrometer), viscosity (Cannon- Fenske viscometer) and calorific value (Bomb calorimeter) of the oil were determined. The fatty acid composition was determined using gas chromatography (GC) with flame ionization detector (FID) using SP2560 fused silica capillary column, 100 m × 0.25 mm × 0.2 µm. Helium gas was used as carrier gas at a flow rate of 1 ml/min. The temperature of injector and detector was maintained at 260 °C and 280 °C respectively. The temperature ramp programming of the oven was 140 °C for 5 min, 140-260 °C at 4 °C/min and held at 260 °C for 15 min. Retention time was verified using FAME mix standard from Sigma- Aldrich.

Biodiesel production from amoora oil

The oil was extracted from the sun dried seeds using mechanical screw expeller (Sardar, India). Filtered oil was and used for biodiesel production studies. A two stage acid- base process was adopted for the production of biodiesel using H_2SO_4 as acid catalyst for pretreatment (acid esterification) and NaOH as base catalyst for transesterification process with methanol. In acid esterification stage, 5 % (w/w) of H_2SO_4 based on FFA and 40:1 methanol to FFA molar ratio were added to the oil. The pretreatment reaction was carried out at 60 °C for 1 h in a 2 L round bottom flask fitted with a reflux condenser, a thermometer and a sampling port with constant stirring on a magnetic stirrer. The temperature was maintained by using an on-off controller. The reaction mixture was then subjected for settling in a separating funnel for 1 h. The upper layer rich in water, methanol and acid fraction was discarded and lower layer was taken for transesterification reaction in the second stage. The acid value of the treated oil was determined by acid - base titration.

The base catalyzed transesterification was carried out in the same laboratory setup. The setup was filled with pretreated oil and heated to required temperature. The amount of NaOH required for neutralization of FFA in the pretreated oil was determined by titration. The NaOH required for neutralization of FFA in addition to 3.5 g/L for triglycerides as catalyst for the reaction was dissolved in methanol to form sodium methoxide (CH_3ONa). Sodium methoxide was added to the preheated oil. The molar ratio of oil to methanol in the range of 1:3, 1:6 and 1:9 was studied. Reaction temperatures were maintained at 40 °C, 50 °C, 60 °C and 70 °C using methanol molar ratio 1:6 to study the effect of temperature on transesterification reaction. Then samples were collected at different intervals and subjected for settling in a separating funnel to form upper biodiesel layer and lower glycerin layer. Glycerin was separated and the biodiesel thus produced was washed two times with water acidified with 0.1 % acetic acid and two washes with regular water. Biodiesel was dried by heating at 120 °C for 20 min to remove the moisture. Thus obtained product was cooled, filtered and then subjected to further analysis. The per cent conversion was determined based on the material balance of the reaction and combined glycerin present in the biodiesel by iodometric titration (AOCS 1991).

Quality analysis of amoora biodiesel

The acid value, saponification value and iodine value were determined by standard methods (Jayaraman 1981).The biodiesel properties were measured as per American society for testing and materials (ASTM) methods as follows: kinematic viscosity (ASTM D445), free glycerin (ASTM D6584), flash point (ASTM D93), copper strip corrosion test (ASTM D130), cloud and pour point (ASTM D2500 and ASTM D97), sulphated ash (ASTM D874).The total FAME content ester in the product was measured by GC analysis according to the EN14103 test method. Oxidation stability was tested as per EN14112 test method. Bomb calorimeter was used to determine the calorific value of the biodiesel. Cetane index was calculated according to Krisnangkura (1986).

Result and Discussion

The observations on flowering and yield pattern in amoora trees planted in avenues were recorded for three consecutive years. Flowering was observed during October to April and fruiting from the end of November till May. The average yield per tree was 20-25 kg in tress aged between 10-15 years (Rajesh et al. 2010). These trees could also be recommended for agro forestry systems as it does not affect the crops. This species has been listed in least concern species by World conservation monitoring centre (www.iucnredlist.org). The trees have the potential to be planted as plantations, avenues as well as stray trees to augment the production. The tree bears fruits for 6 to 7 months in a year, which is advantageous for continuous supply of feed stock for biodiesel production. Since the plant is well adapted to Western Ghats and also to plains, it is advantageous for promoting this tree species as a candidate for biodiesel production.

Qualitative and quantitative analysis of oil

The oil content in seeds was found to be 40 - 44 % which is in agreement with the previous literature on amoora (Sengupta and Mazumder 1976) out of which 35 % oil could be expelled mechanically by using screw expeller. The biochemical properties of the oil extracted from *Aphanamixis polystachya* have been listed in Table 1. Saponification value and iodine value of the amoora oil were found to be 171.1 mg KOH/g of oil and 107 g I_2/100 g oil respectively. The iodine value is the measure of unsaturated fatty acids in the oil. The results show that amoora is a potential source of oil and hence the seeds could be viable option for biodiesel production.

Table 1: Biochemical properties of the oil from *Aphanamixis polystachya*

Properties	Values
Oil percentage	40 – 44 %
Acid value	9.24 mg KOH/g
FFA	4.62 %
Saponification value	171.1 mg KOH/g
Iodine value	107 g I_2/100g
Color	Yellowish brown
Viscosity	44.9 mm^2/s
Density	919 kg/m^3
Calorific value	35.8 MJ/kg
Avg. molecular weight of fatty acids	291

Fatty acid profile

The fatty acid profile of oil using GC is shown in Fig. 1. The fatty acid composition of the oil is given in Table 2. The fatty acid composition was in agreement with the studies carried out by Sengupta and Mazumder (1976) where the oil composition were C16:0 (24.8 %), C18:0 (12.4 %), C18:1(20.9 %) and C18:2 (28.5 %). The small variation in the fatty acid content could be attributed to the result of the environmental factor where the tree is grown. The amoora oil was found to have 36.57 % linoleic acid (C18:2) which constitutes about 1/3rd of the total fatty acids, followed by palmitic acid (C16:0) - 22.095 %, oleic acid (C18:1) - 19.07 %, stearic acid (C18:0) - 13.56 % and eicosenoic acid (C20:1) - 7.04 %. It was found that the oil contains 63.4 % unsaturated fatty acids. The higher unsaturation and oleic acid content implies the suitability of the oil in biodiesel production which improves the cold properties of the fuel.

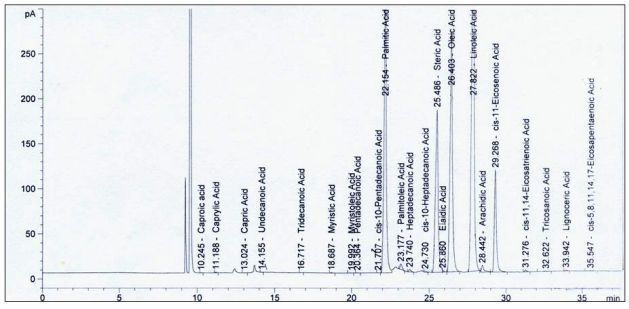

Figure**1**: Gas chromatogram of fatty acid profile in amoora oil

The fatty acid composition of amoora oil has been compared with other non edible oils; pongamia, mahua, neem, palm and jatropha (Table 2). Even though amoora and neem (*Azadirachta indica*) belong to family, Meliaceae, there were significant differences in fatty acid composition. The saturated fatty acids of amoora (C16:0 - 22.095 %, C18:0 - 13.56 %) were comparable with neem (C16:0 – 14.9 %, C18:0 –14.4 %), (Mohibbe et al. 2005) where as there was a large difference in unsaturated fatty acids. The linoleic acid (36.57 %) was higher than oleic acid (19.07 %) in amoora compared to neem which had lower linoleic acid (7.5 %), than oleic acid (61.9 %). The composition of amoora oil showed higher linoleic acid compared to the oleic acid whereas other oils viz. pongamia, mahua, neem, palm and jatropha showed higher oleic acid compared to linoleic acid as reported in previous studies (Ghadge and Raheman 2006; Gubitz et al. 1999; Mohibbe et al. 2005; Karmee and Chadha 2005).

Table 2: Comparison of fatty acid composition of the oil from different non edible oils

Fatty acids	Average percentage					
	Amoora[1]	Pongamia[a]	Mahua[b]	Neem[c]	Palm[d]	Jatropha[d]
Myristic acid (C14:0)	--	--	--	--	0.5-6	0-0.1
Palmitic acid (C16:0)	22.095	3.7-7.9	16-28.2	14.9	32-45	14.1-15.3
Palmitoleic acid (C16:1)	0.45	--	--	--	0.8-1.8	0-1.3
Stearic acid (C18:0)	13.56	2.4-8.0	20-25.1	14.4	2-7	3.7-9.8
Oleic acid (C18:1)	19.07	44.5-71.3	41-51	61.9	14-43	34.3-45.8
Linoleic acid (C18:2)	36.57	10.8-18.3	8.9-13.7	7.5	5-11	29.0-44.2
Linolenic acid (C18:3)	--	--	--	--	Trace	0-0.3
Arachidic acid (C20:0)	0.50	2.2-4.7	0-3.3	1.3	Trace	0-0.3
Behenic acid (C22:0)	--	--	--	--	--	0-0.2
Eicosenoic acid (C20:1)	7.04	9.5-12.4	--	--	--	--

[1] Present study; [a] Karmee et al. 2005; [b] Ghadge and Raheman 2006
[c] Mohibbe et al. 2005; [d] Gubitz et al. 1999

Biodiesel production

Acid esterification

The acid value of the oil was found to be 9.24 mg KOH/g of oil. Higher acid value indicated the presence of high amount of FFA (4.62 %), which may be due to the hydrolysis of triglycerides. The higher FFA content may lead to formation of soap in the presence of moisture during transesterification process. Hence acid pretreatment was required prior to transesterification process for biodiesel production to reduce FFA below 3 % (Sulistyo et al. 2009; Dennis et al. 2010). During acid treatment the FFAs are converted into the biodiesel by which the acid value of the oil decreases. There was drastic decrease in the acid value of the oil from 9.24 mg KOH/g to 1.8 mg KOH/g of oil (0.9 % FFA) over a period of 60 min reaction time (Fig. 2).

Transesterification process

The FFA level was 0.9 % which indicated that 1.28 g of NaOH required for the neutralization of the FFA present in 1 L oil. Transesterification of pure triglycerides require 3.5 g NaOH **(**Dennis et al. 2010; Pelly and Michael 2009; Slinn and Kendall 2009). Hence total 4.78 g NaOH was dissolved in required amount of methanol to form sodium methoxide was added to the pretreated oil as catalyst mixture.

Effect of oil to methanol molar ratio

In transesterification process, methanol to oil ratio is one of the important parameter affecting the biodiesel conversion. In the present study 1:3, 1:6 and 1:9 molar ratios were investigated at 60 °C temperature. Fig. 3 shows the effect of different molar ratio on

biodiesel conversion. A gradual reduction in density and viscosity was observed (Fig. 4) as the conversion process advanced. Molar ratio of 1:6 was found to be better than the others with maximum conversion of 97.8 % in 1 h reaction time. The previous study on

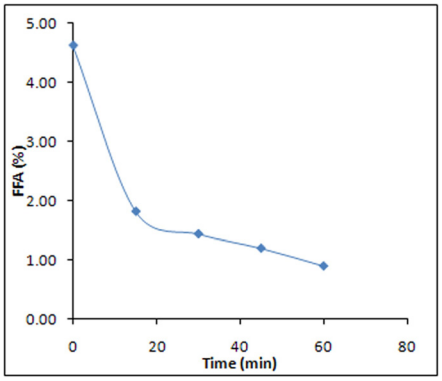

Figure 2: Reduction in FFA content by esterification using H_2SO_4 (5 %) and methanol (40:1 methanol to FFA molar ratio)

pongamia reported similar results but the maximum conversion was 97 % after 3 h reaction time at 65 °C with KOH as catalyst (Meher et al. 2006a). The rate of the reaction was very high for the first 15 min where the conversion reached to 96.4 % and later no noticeable increase in conversion was detected. It was also observed that after 90 min there was slight decrease in the conversion which indicated that there might be backward reaction favored by the formation of glycerol. Similar observations were recorded by Sulistyo et al. (2009) where the conversion reached to 96 % after 15 min. Although the stoichiometric equation shows 1:3 molar ratio of methanol for reaction, the conversion was found to be less compared to 1:6 molar ratio (Knothe et al. 2005; Meher et al. 2006b). At higher ratio (1:9), initially the conversion was high but as the reaction progressed, the conversion was decreased; which may be attributed to glycerol formed during the reaction that favored the backward reaction. The excess methanol may act as solvent for the methyl esters and glycerol, that enhance the contact between the molecules and favor the backward reaction.

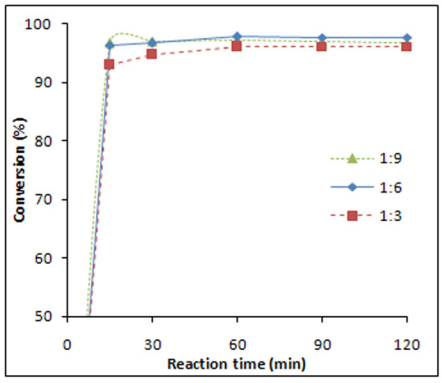

Figure 3: Effect of oil to methanol molar ratio on transesterification of amoora oil at 60 °C

Effect of temperature

The effect of temperature on the transesterification reaction is shown in Fig. 5. The results showed that increase in temperature increase the reaction rate. The optimum temperature was found to be 60 °C. Highest conversion of 97.8 % was observed at this temperature. Further increase in temperature did not increase the

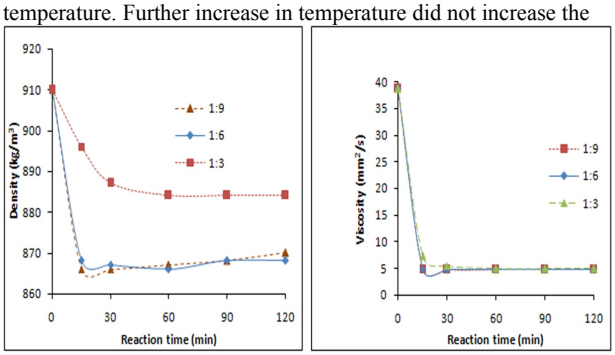

Figure 4: Reduction in viscosity and density of amoora by transesterification reaction at 60 °C

rate of reaction considerably. Higher temperature increased the evaporation of methanol, hence the rate of reaction was found to decrease (Meher et al. 2006a). The reaction temperature (60 °C) slightly less than the boiling point of methanol was optimum for transesterification reaction.

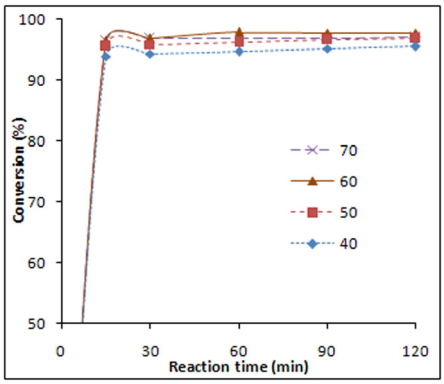

Figure 5: Effect of temperature on transesterification of amoora oil using 1: 6 oil to methanol molar ratio

Properties of amoora biodiesel

The maximum biodiesel yield was found to be 96 % (v/v) for 1 h reaction time at 60 °C temperature and 1:6 oil to methanol molar ratio. The properties of amoora biodiesel have been tabulated (Table 3). The result showed improvement in properties such as density (866 kg/m^3), viscosity (4.67 mm^2/s) and calorific value or high heating value (38.3 MJ/kg). There was substantial decrease in the viscosity of oil from 44.9 mm^2/s to 4.67 mm^2/s by transesterification process. There was a reduction in iodine value of biodiesel from 107 to 93 g I_2/ 100 g by transesterification which was comparable with the previous results where there was reduction in iodine value of different vegetable oil by transesterification process (Dobromir et al. 2007).The reduction of iodine value may be due to the change in the molecular structure from triglycerides to the FAME. The total FAME content (ester content) was 99.8 % which shows the completion of the transesterification process. The copper strip corrosion test was found to be No. 1a, which showed that the inorganic acids are within the limits (No.3) in the biodiesel produced; hence amoora biodiesel is safe to be used in the automobile engines since the corrosion of the fuel system of vehicle is negligible. The cloud and pour point of the biodiesel were 12 °C and 6 °C respectively. The result indicated that biodiesel could not be used at low temperature. But by adding anti-freezing agents the cold properties of biodiesel may be improved. The oxidation

stability of amoora biodiesel was 2 h. The amoora biodiesel failed in oxidative stability test; the reason may be biodiesel obtained from the oil are easily attacked by the enzyme and degrade easily. The oxidative stability of the fuel could be enhanced by adding antioxidants. The properties of amoora biodiesel were on par with biodiesel from pongamia, mahua, jatropha and petroleum diesel (Table 3). The amoora biodiesel was found to be within the American (ASTM D6751) and Bureau of Indian Standards (BIS) (ISO15607) specifications.

Table 3: Comparison of properties of biodiesel from amoora with pongamia, jatropha and mahua

Properties	Values						
	Amoora[a]	Pongamia[b]	Mahua[b]	Jatropha[c]	ASTM D6751	BIS (ISO 15607)	Diesel[d]
Density (kg/m^3)	866	876	880.0	880.3	--	860-900	840.0
Viscosity (mm^2/s)@40°C	4.67	9.6	3.98	4.328	1.9-6.0	2.5-6.0	3.12
Acid value (mg KOH/g)	0.34	0.31	0.41	0.27	0.5 max	0.5 Max	0.02
Iodine value (g I_2/100g)	93	--	--	62	--	120 Max	--
Saponification value (mg KOH/g)	171.1	--	--	--	--	--	--
FAME (%)	99.8	--	--	98.6	--	96.5 Min	--
Free glycerin (%)	Nil	--	--	Nil	0.02 Max	0.02 Max	--
Copper strip test	1a	--	--	1a	3	Class1	--
Cloud point (°C)	12	--	--	--	--	--	<-1
Pour point (°C)	6	7	6	-1	--	--	--
Flash point (°C)	154	187	208	>130	130 Min	120 Min	79
Oxidation stability (h)	2	--	--	3.4	--	6 Min	--
Sulphated ash (%)	0.004	0.01	0.01	--	0.02	0.02 Max	0.01
Cetane index	55	--	--	--	47 min	51 min	46.0
Calorific value (MJ/kg)	38.3	36.12	37	39.6	--	--	44.96

[a]Present Study, [b]Ghadge and Raheman 2006; [c]Nakpong and Wootthikanokkhan 2010; [d]Ikwuagwu et al. 2000

Conclusion

Aphanamixis polystachya was investigated as non-edible source of biodiesel. The study showed that amoora oil has great potential to be used as one of the feed stocks for biodiesel production, mainly owing to its higher oil content and yield potential. A two stage acid-base process was needed for biodiesel production from amoora oil containing higher FFA. The quality of the biodiesel was found to be comparable with the ASTM and Indian standards. Hitherto, amoora tree, generally grown for medicinal and ornamental purpose could now be promoted as a potential biofuel crop.

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