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Effect of *Rhizoclonium grande* Saturated Fatty Acid Ethyl Esters on Physicochemical and Fuel Properties of *Jatropha curcas* Biofuel

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JMK, CS and RM designed the study and JMK, JOO and TT wrote the protocols. Authors JMK and JOO wrote the first draft of the manuscript, managed the literature searches, analyses of the study, performed the structural modelling of abstract, discussion and the conclusion. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The effect of *Rhizoclonium grande* saturated fatty acid ethyl esters on physicochemical and fuel properties of *Jatropha curcas* biofuel was assessed.
Study Design: Probability and purposeful sampling methods were employed. Randomized block design comprising of three blocks, *Rhizoclonium grande* and *Jatropha curcas* oils, the ethyl esters and their blends. Each sample from a block was subjected to several treatments with three replications per treatment.
Place and Duration of Study: the Technical University of Mombasa, Jomo Kenyatta University of Agriculture and Technology, Government Chemist and Kenya Pipeline Laboratories in Mombasa between February 2012 and September 2014.

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Methodology: Algae was collected from Shimoni in Kwale, Shelly beach, Jamvi la Wageni in Likoni and English point in Mombasa to obtain algae oil. *J. curcas* seeds were collected from Shimba hills, Kwale. Transesterification of *J. curcas* and *Rhizoclonium grande* oil was carried out separately followed by GC-MS characterisation of the ethyl esters. Ethyl esters from *J. curcas* oils (JOFAEE) were blended with algae oil FAEE (5-25%) to obtain JAB. Physico-chemical and fuel properties of JAB were assessed. Statistical analysis was done using STATA/SE 13.0 at 95% confidence level ($P<0.05$) two-tailed.
Results: *Rhizoclonium grande* produced 6.72 - 10.46 % v/w oil. From GC-MS analysis the algae oil fatty acid esters contained pentadecanoic acid ethyl ester, dodecanoic acid ethyl ester, tetradecanoic acid ethyl ester and hexadecanoic acid ethyl ester. There was an observed significant difference in calorific value ($P=0.03$), kinematic viscosity ($P=0.001$), pour point ($P=0.001$) and cloud point ($P=0.001$) between JO FAEE and JAB samples attributed to the presence of algae oil saturated FAEE. The JAB showed no significant difference in fuel properties with standard biodiesel B100 ($P>0.05$).
Conclusion: From the findings, the physicochemical and fuel properties of biofuel from *J. curcas* can be improved by the use of saturated FAEE from *R. grande* for sustainability.

Keywords: Miscibility; Biofuel blend; Physico-chemical properties; Fuel properties.

1. INTRODUCTION

There are various current issues with biofuel products and use discussed in popular media and scientific journals such as food versus fuel debate [1,2]. There is a need for non-food crops biomass such as *J. curcas* L. and carbon dioxide scrubbers such as algae. It is claimed that algae can produce up to 30 times more energy per acre than land crops such as soya bean hence more sustainable [3]. *J. curcas* L. has high quantity 53–57% non–edible oil but still not sustainable [4]. The main renewable feedstock that has the potential to replace petroleum diesel completely without adversely affecting food supply chain and other crop products is algal oil [5,6,7,8].

Most algae oil contain palmitic acid, palmitoleic acid, stearic acid, oleic acid, octadecadienoic acid, octadecatrienoic acid, giving a total unsaturated fatty acid esters content around 85% [9]. Additionally it contains 0.01-0.02% moisture and 0.45-1.75% free fatty acid (FFA) [10]. Notably, FFA is always below 2% [11]. Based on these data, it is assumed that triolein and Oleic acid represent the bulk of algae oil [12]. Endalew et al. [13] tested lithium CaO doped catalysts for biodiesel production from high acidity jatropha oil but, as expected, they found that both CaO an d Li-doped CaO catalysts led to the production of large amounts of soaps due to a high level of FFA. Puna et al. [14] tested lithium modified lime catalysts for biodiesel production from rapeseed oil with a high yield of >94%. Generally, the mechanism of the base-catalysed transesterification of vegetable oils involves four steps. The first step is the reaction of the base with the alcohol, producing an alkoxide and the protonated catalyst. The second step is the nucleophilic attack of the alkoxide at the carbonyl group of the triglyceride generating a tetrahedral intermediate [15]. The third step involves the formation of the alkyl ester and the corresponding anion of diglyceride. The final step involves deprotonating the catalyst, thus regenerating the active species, which is now able to react with a second molecule of the alcohol, starting another catalytic cycle [16]. Transesterification of triglycerides using ethanol from *Rhizoclonium grande* can be used which is more sustainable and cheaper than synthetic methanol [17]. Diglycerides and monoglycerides are converted by the same mechanism to a mixture of alkyl esters and glycerol. Biodiesel standards such as of European EN 14214, Germany‘s DIN 51606 and America‘s US ASTM D-6751-02 have been used to test the quality of biofuels [18,19].

A biofuel blend of *Jatropha curcas* with algae saturated fatty acid alkyl esters would improve sustainability since algae can be easily cultured and easily produced. Biodiesel has advantages over petroleum diesel such as increasing lubricity and reduction of exhaust emissions [20].

This work seeks to assess the effect of *Rhizoclonium grande* saturated fatty acid ethyl esters on physicochemical and fuel properties of *Jatropha curcas* L. biofuel in order to produce a more sustainable quality biofuel.

2. MATERIALS AND METHODS

2.1 Sample Collection

Macroscopic and filamentous marine algae species *Rhizoclonium grande* from different sampling sites namely Shimoni shores (SS) in Kwale County South Coast, Shelly Beach (SB), in Likoni South Coast, Jamvi la Wageni (JW) in Likoni South Coast, English point (EP) near Kenya Marine and Fisheries Research Institute (KMFRI) Mombasa North coast and Tudor Creek (TC) near Kenya Meat Commission on Makupa Causeway were randomly sampled and separated from the growing medium [21]. It was identified by a taxonomist from KMFRI, collected and put in 5 kg polythene sample bags. The samples were kept in cold boxes and transported to Technical University Laboratories for further treatment. The algae species samples were dried under a shade for three days (Plate 1) then they were ground to a powder using Hamilton Beach commercial blender.

J. curcas seeds were collected from selected farms FA, FB, FC, FD and FE in Shimba Hills, Kwale County one of the major Jatropha growing area. The farms were randomly sampled by snow balling and the seeds were bought in 50 kg gunny polypropene bags. The husked seeds (Plate 2) were transported to the Technical University of Mombasa where they were dehusked using a locally fabricated dehusker. The dehusked seeds were preserved in polythene bags in an air-conditioned room at temperature 25ºC awaiting extraction.

2.2 Oil Extractions

In extraction 350 g of the ground marine algae *Rhizoclonium grande* were placed in 1L Erlenmeyers flasks then was added 700 mL of hexane (boiling point range 63-70ºC), obtained from Ranchem Chemicals. The mixture was kept in a cupboard in Chemistry laboratory at the Technical University of Mombasa for three days. The mixture was then filtered by vacuum filtration method into a 500 mL Buchner flask. The solvent was recovered using a vacuum evaporator model ROVA-3L at 50ºC with a negative pressure of 450 mmHg. The algae oil obtained was kept open for a further three hours to evaporate all the solvent in a fume cupboard then measured using 10 mL and 100 mL measuring cylinders. Traces of chlorophyll were removed from algae oil using activated charcoal over a filter paper by vacuum filtration. The algae oils were transferred into glass sample bottles and labelled. The oil (AO) was then analysed for oil physicochemical properties using Association of Official Analytical Chemists (A.O.A.C) 17th edition, 2000, official methods [22] and ASTM procedures.

J. curcas oil (JO) was extracted using fabricated cold oil press and n-hexane as the solvent and also industrial blender for grinding [17]. The oil was allowed to settle then filtered using vacuum filtration. For the solvent extracted oil from the cake, the solvent was recovered by vacuum evaporator model ROVA-3L at 50ºC with a negative pressure of 450 mmHg and reused. The amount of oil in both cases was measured using a 100 mL measuring cylinder and kept in a refrigerator at 4ºC and later analysed similarly as for algae oil.

2.3 Transesterification Blending and Analysis of Fatty Acid Ethyl Esters

Dry ethanol prepared from *Rhizoclonium grande* L. biomass was used for transesterification [17, 23]. Dry potassium hydroxide was reacted with the bioethanol to produce the ethoxide ions mixture in a ratio of 1: 10. The mixture was added to *Rhizoclonium grande* L and *Jatropha curcas* L oils separately in a ratio of 3:10 ethoxide ion solution from the bioethanol to oil preheated at 65 ^{0}C [24] with constant stirring using a mechanical stirrer at 200 rpm for two hours [25] to produce fatty acid ethyl ester biofuels. The ethyl esters were then separated from the glycerol by centrifugation using centrifuge Model Neofuge 15R at 10,000 rpm for 15 minutes followed by decantation, washing with distilled water and drying with anhydrous sodium sulphate.

The ethyl esters from algae oil were analysed by GC-MS analysis to ascertain composition. The crude ethyl esters were then analysed for physicochemical and fuel properties using ASTM standard test methods.

Each of the fatty acid ethyl esters (FAEE) was analysed separately before blending in different proportions from 0,5,10,15,20 to 25% of FAEE from algae oil. Again analysis of the *J. curcas* and *Rhizoclonium grande* L FAEE (JAB) blends was carried out to determine the effect on miscibility, physicochemical and fuel properties according to procedures based on ASTM D4682 and D6751.

Plate 1. *Rhizoclonium grande* after drying

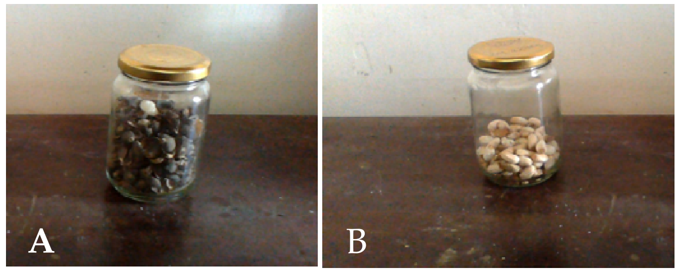

Plate 2. *Jatropha curcas* seeds. A. Husked seeds B. Dehusked seeds

Plate 3. *Jatropha curcas* oil

Plate 4. *Rhizoclonium grande* Algae oil

2.4 Data Analysis

Exploratory data analysis and standard statistical analyses were done using the statistical software package STATA, version SE 13 (STATA/SE13 © 1985-2013 StataCorp LP). Three independent replicates were used per analysis and the results were expressed as mean values ± standard deviation of the mean. The General Linear Models (GLM) procedure was used to analyze the effect of the percentage of *Rhizoclonium grande* algae FAEE on fuel properties of ethyl ester obtained from *Jatropha curcas* oils. When the means of the GLM were statistically different, these means were further compared to the control and the experimental groups using pairwise comparisons, t-tests and ANOVA. Significance was based on $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Volume of Algae Oil Extracted from *Rhizoclonium grande*

The table below shows the volume of algae oil extracted from *Rhizoclonium grande* using hexane from four sampling sites. From Table 1 Jamvi la Wageni recorded the highest yield of algae oil 36.61±1.276 mL (10.46±0.365 $\%^{v}/_{w}$) and Shelly beach recorded the lowest 23.50±0.481 (6.72±0.130 $\%^{v}/_{w}$).

Table 1. Volume of algae oil extracted using hexane

Sample site	Algae biomass (g)	Vol. of hexane (mL)	Vol. of algae oil (ml)	yield $\%^{v}/_{w}$
Tudor creek	350	700	28.90±0.395	8.26±0.114
Shimoni shores	350	700	30.36±0.319	8.68±0.084
Shelly Beach	350	700	23.50±0.481	6.72±0.130
Jamvi la wageni	350	700	36.61±1.276	10.46±0.365

3.2 Ethyl Esters Yield from *Rhizoclonium grande* Algae Oil

Table 2 shows the percentage yield of the ethyl esters was highest in algae oil from Jamvi la Wageni (62%) and the least was from Tudor creek (57%). The values are in agreement with those in literature for microfilamentous algae of 68% [26]. The relatively lower yield values of alkyl esters compared to methyl esters could be attributed to the difficulty experienced in separation and isolation of ethyl esters and glycerol. Deactivation of the catalyst due to the presence of glycerol could also be a contributing factor to lower yield [14].

3.3 Yield of Ethyl Esters from *Jatropha curcas* L. Oil

The percentage yield of the ethyl esters from five different fields was found to be 59.0 ± 2.16% [17]. The relatively lower yield values of alkyl esters compared to methyl esters [27] could be attributed to incomplete separation and isolation of ethyl esters, glycerol and soap, though it may be more desirable to make ethyl esters because ethanol can be derived from renewable sources [17,25].

3.4 Physicochemical and Fuel Properties of Ethyl Esters from *Rhizoclonium grande* Oil

From Table 3 the algae ethyl esters from all the sample sites had properties acceptable by ASTM D 6751. The mean values obtained are in agreement with those obtained from different species of algae [28]. According to the findings on two algae species, *C. vulgaris* and *R. hieroglyphicum* the biodiesel produced was analyzed for kinematic viscosity (4.9 and 5.0 mm^2/s), flash point (160 and 156ºC), specific gravity (0.91 and 0.914 g/mL), cetane number (51 and 49) for *C. vulgaris* and *R. hieroglyphicum* respectively. Properties of the biodiesel were compared with ASTM standard (ASTM D 6751) and it was found to be quality biodiesel.

3.5 GC-MS Spectra of *Rhizoclonium grande* L. Ethyl Esters

From the GC-MS spectrum (Figure 2) the composition of the ethyl ester mixture include Pentadecanoic acid ethyl ester Rt = 6.376 (1), Dodecanoic acid (lauric acid) ethyl ester Rt = 8.168 (2), Tetradecanoic acid (myristic acid) ethyl ester Rt = 9.593 (3) and hexadecanoic acid (palmitic acid) ethyl ester Rt =10.168 (4). Others not identified are in low quantities at Rt =10.943 (5), Rt =11.051 (6) and Rt=12.035 (7). The composition of fatty acid ethyl esters shows that *Rhizoclonium grande* mainly contain saturated fatty acids lower in chain length than found by Meng et al. [9]. Saturated fatty acids are less susceptible to auto-oxidation.

3.6 Physicochemical and Fuel Properties of Ethyl Esters from *Jatropha curcas* L. Oil

Jatropha ethyl esters from all the sample sites had physicochemical and fuel properties [17] acceptable by ASTM D 6751 (Table 4). The mean values obtained are comparable with those obtained for *Jatropha curcas* methyl esters from various publications [15,29] and were comparable to the ASTM standard biodiesel B100 [30] (Table 4) showing that they were within the specifications.

3.7 Physicochemical and Fuel Properties of *Rhizoclonium grande* L. And *Jatropha curcas* L. Fatty Acid Ethyl Esters Blends

Miscibility studies showed that 20% AO FAEE blended with JO FAEE was completely miscible. Physicochemical and fuel properties of the biofuel blends investigated (Table 4) showed there was an observed significant difference

Table 2. % yield of ethyl esters from *Rhizoclonium grande* algae oil sample

Algae oil sample	The volume of Algae oil (mL)	Mass of Potassium hydroxide (g)	The volume of dry ethanol (mL)	Mean ±SD Volume of ethyl esters (mL)	Mean ±SD %yield of ethyl esters
AO1	10	0.3	3	5.7 ±0.06	57±0.6
AO2	10	0.3	3	5.8±0.00	58±0.0
AO3	10	0.3	3	5.9±0.10	59±1.0
AO4	10	0.3	3	6.2±0.06	62±0.6

AO1 Tudor creek, AO2 Shimoni shores, AO3 Shelly beach and AO4 Jamvi la wageni samples

Table 3. Physicochemical and fuel properties of ethyl esters from algae oil

Algae Ethyl ester (FAEE) sample	Physicochemical and fuel properties (mean values)							
	Density kg/m^3 ASTM D1298 at 20°C	Calorific value (MJ/Kg) ASTM D4809	Kinematic viscosity at 40°C (Cst) ASTM D445	Flash point (°C) ASTM D93	Cetane index ASTM D4737	Pour point (°C) ASTM D97	CFPP ASTM D6371	Cloud point (°C) ASTM D2500
FAEE TC	910	33	4.7	153	48	-6	-3	-2
FAEE SS	912	34	4.9	154	50	-5	-2	-1
FAEE SB	909	32	4.7	152	49	-6	-3	0
FAEE JW	913	34	5.1	155	50	-5	-1	1

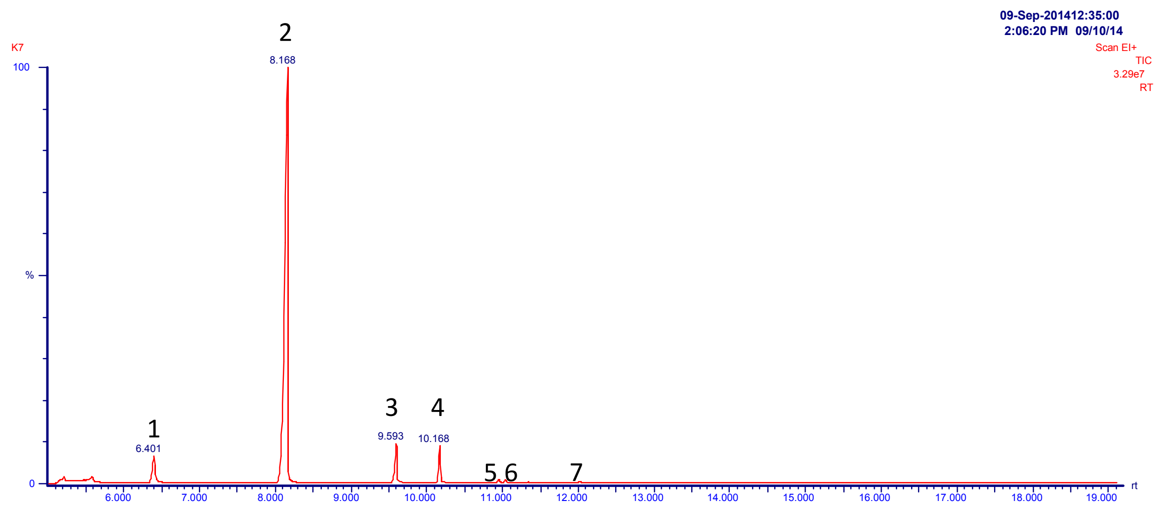

Fig. 1. GC-MS analysis of *Rhizoclonium grande* ethyl esters

Table 4. Miscibility physicochemical and fuel properties of ethyl esters from algae and Jatropha fatty acids blends

JAB samp	algae FAEE %Comp. in JAB and miscibility ASTM D4682-87	Physicochemical and fuel properties (mean values)							
		Density kg/m^3 ASTM D1298 at 20°C	Calorific value (MJ/Kg) ASTM D4809	Kinematic viscosity at 40°C (cSt) ASTM D445	Flash point (°C) ASTM D93	Cetane index ASTM D4737	CFPP ASTM D6371 (°C)	Pour point (°C) ASTM D97	Cloud point (°C) ASTM D2500
JAT FAEE	0	874.8	40.89	5.410	182.6	64.1	-2	-9	-3
JAB 1	5 miscible	864.5	41.38	4.760	181	63.0	-2	-6	1
JAB 2	10 miscible	865.0	41.35	4.765	182	63.3	-2	-6	1
JAB 3	15 miscible	865.1	41.35	4.780	182	63.2	-1	-6	2
JAB 4	20 miscible	865.2	41.34	4.783	183	63.3	-1	-6	1
JAB 5	25 slightly miscible	865.9	41.34	4.795	183	63.3	-1	-5	2
B100	-	800-900	report	1.9-6.0	130 min	47 min	report	-10 to 12	report

B100 standard biodiesel, JAT FAEE Jatropha fatty acid ethyl esters from the fields FA-FE (mean values) in density, calorific value, kinematic viscosity, pour point and cloud point between JO FAEE and JAB samples. This was according to the Statistical analysis of data obtained using scientific data analysis software (STATA/SE 13.0) at 95% confidence level ($p<0.05$) two-tailed. The addition of algae FAEE (20%) had a significant effect in density, kinematic viscosity, pour point and CFPP this may be attributed to the lower molecular weight composition of FAEE of *Rhizoclonium grande* L (Fig. 1) compared to those of *Jatropha curcas* L [17]. There was an observed significant difference in calorific value ($p=0.03$), kinematic viscosity ($p=0.001$), pour point ($p=0.001$) and cloud point ($p=0.001$) between JO FAEE and JAB samples attributed to the presence of algae oil saturated FAEE. According to the standard biofuel B100 (Table 4), the physicochemical and fuel properties of JAB are within the allowable values (ASTM D 6751).

4. CONCLUSION

Rhizoclonium grande saturated FAEE can be used to blend with *Jatropha curcas* FAEE to improve sustainability, physicochemical and fuel properties especially density, calorific value, flash point and kinematic viscosity. There is no significant difference in physicochemical and fuel properties of the 20% JAB blend and standard biodiesel B100.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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