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EFFECTS OF FREE FATTY ACIDS (FFAS) ON THE QUALITY OF *Euphorbia tirucalli* (L.) OIL EXTRACTED FROM DIFFERENT STEM DIAMETERS IN DIFFERENT AGRO-ECOLOGICAL ZONES OF TANZANIA.

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ABSTRACT
Plant oils having lower levels of percent Free Fatty Acids (%FFAs) moieties offer particular potentials in evaluation of qualities of liquid biofuels, because they are associated with reduction of atmospheric pollution relative to petroleum diesel fuel. The present study examined effects of FFAs on the quality of *Euphorbia tirucalli* (L.) oil extracted from different stem diameters such as 20cm, 30cm, 40cm, 50cm, 60cm, 70cm and 80cm. Soxhlet extraction method was used in oil extraction. Acid Values (AVs) were determined by standard methods ASTM D 664 and the Cd 3d-63 of the American Society for Testing and Materials and the American Oil Chemists Society respectively. The AVs so obtained were used to calculate the amount of FFA contents in *E. tirucalli* oil as percent oleic acid. One-way ANOVA with the Minitab software v.16 was used to test for differences in %FFAs. The %FFA contents of the oil were compared with the international quality biofuel standard ASTM D 6751 (0.020max). Results showed that the quality of oil which was expressed in terms of %FFAs of oil ranged from 4.57 $\pm$ 0.02 at 20cm to 4.72 $\pm$ 0.02 at 40cm, 3.61 $\pm$ 0.10 at 40cm to 4.42 $\pm$ 0.10 at 30cm and 2.45 $\pm$ 0.07at 30cm to 3.06 $\pm$ 0.07 at 60cm stem diameters from Dodoma, Mbeya and Dar es Salaam agro-ecological zones in the same order. These results indicated low quality of oil since the entire %FFAs were above 0.020max%, not complying with the ASTM D 6751 standard. Also the obtained %FFAs of oils differed significantly ($P \leq 0.05$) among different stem diameters and agro-ecological zones. The study concluded that the quality of oil (%FFAs) was low and found to decrease from low to high stem diameters of *E. tirucalli* in different agro-ecological zones.

Keywords:
E. tirucalli, Free Fatty Acids (FFAs), quality of oil, agro-ecological zone, stem diameters, stem bark

INTRODUCTION
Currently the world is increasing attention on biofuel production in order to reduce overdependence on fossil fuels (Rajesh *et al*., 2008). In an effort to the advancement of this enterprise, Eshel *et al.,* (2010) warned on utilization of non-food sources in order to avoid hunger which may result from using food crops as sources of biofuel. Thus, the emphasis of liquid biofuel production was much given on non-food plant sources. In Sub-Sahara African countries, non-food plant based liquid bio fuels can be obtained from a variety of sources including physic nut (*Jatropha*) and the latex producing plant species such as milkweed (*Calotropis procera*) and the African Milk bush (*Euphorbia tirucalli*) (FARA, 2008; Kumar, 2009). *E. tirucalli* tree, the focus of this study, is one among the best alternative non-food crops that can be used as sources of liquid biofuels (Photi, 2011). The plant was popularized as a source of liquid biofuel by Calvin in the late 1970s to early 1980s and its biofuel properties were researched on by Petrobras, the Brazilian national petroleum company in the 1980s (Calvin, 1979; Buchanan *et al,* 1979; Calvin, 1980; Bagby *et al.*, 1983;Van Damme, 2009). *E. tirucalli*, like other latex exuding plant species can produce low-molecular-weight organic liquids, several of which have been proposed for use as transportation fuels for vehicles driven by spark or compression ignition engines (Van Damme, 2009).
Several authors reported that the latex of *Euphorbia tirucalli* contains forms of hydrocarbon molecules including terpenoid constituents (Calvin, 1980) with the major components being triterpenes (Biesboer *et al.,* 1979; Yamamoto *et al.,* 1981). These forms of molecules in the *E. tirucalli* latex have a molecular size distribution very much like that in petroleum, so that after separating them from water, they can be refined into gasoline through cracking (Nielsen et al., 1977; Calvin, 1980; Depeyre *et al*., 1994; Gildenhuys, 2006). Apart from the potential energy value of *E. tirucalli,* the plant has also been reported to be one of the highly valued species for live fences in Tanzania.

Many different plant oils including *E. tirucalli* oil have been tested for their suitability as liquid biofuels particularly biodiesel to fuel direct-injection engines or other diesel-powered machines (Buchanan *et al*, 1979; Calvin, 1980; Bagby *et al.*, 1983;Van Damme, 2009). Nevertheless, several problems may arise when the raw plant oils are used as liquid biofuel in vehicle engines or other moving and stationary machines. The main problem which has major effects on fuel properties is higher percentage of unsaturated free fatty acids (FFAs) (Hu, *et al.* 2008; Greenwell, et al., 2010). Other problems are higher viscosity and lower volatility of the oil when compared with the corresponding properties of petroleum diesel fuels (Furrer *et al.*, 1989; Hill *et al.*, 1984). Higher unsaturated FFA contents can affect oil properties which include declining in oil induction time (oxidative stability), excessive corrosion and engine deposits, particularly in the fuel injectors (Furrer *et al.*, 1989). Another effect due to higher FFA contents in oil occur during biodiesel production in which FFAs can react with the alkali catalyst to form soap instead of biodiesel (Warabi, *et al*., 2004). This may reduce the amount of free catalyst thereby reducing the speed of the transesterification reaction (Jitputti *et al.*, 2006). Soap formation can restrain separation of ester from the glycerin which delays the reaction. Also, because the soap is not needed, more soap formation might reduce the amount of biodiesel produced (Lopez *et al.*, 2007). In order to overcome these problems, many researchers had recommended the use of transesterified oils that can reduce the amount of FFAs and viscosity of the oil to the highest degree. This transesterified vegetable oil is called biodiesel (Liu *et al.*, 2007). During biodiesel production through transesterification process, FFAs are produced when some triglycerides in oils are broken down and separated from the glycerol molecule. Furthermore, oil extracts isolated from latex and stem of *E. tirucalli* grown in Madagascar have been reported to contain a variety of conjugated unsaturated fatty acids as acyl groups that are highly sensitive to undergo autoxidation (Fürstenberger and Hecker, 1977). It is due to these reasons that percent (%) FFA becomes one among important parameters for the assessment of quality of *E. tirucalli* oil. Thus, for liquid fuel oils such as biodiesel produced through the transesterification process and the raw plants oils including *E. tirucalli* oil having FFAs must conform to the preferred worldwide standard fuel properties (ASTM, 2002; Suwannakarn, *et al.*, 2007). The American Society for Testing and Materials (ASTM D 6751) and European (EN 14214) are worldwide preferred standard fuel properties developed by the United States of America (USA) and the Europeans (EU) respectively (ASTM, 2002). These standard specifications were developed in order to ensure proper performance and maintenance of oil quality. Both the US and EU standards recommend FFA levels of less than 0.020 % (ASTM, 2002). Moreover, plant oils investigated for their suitability as liquid biofuel are those which occur abundantly in the country of testing (Wang Shi-chi and Huffman, 1981; Bagby, 1996). Therefore, in the prevailing local environmental conditions of Tanzania, it was crucial to study the effects of FFAs on the quality of *Euphorbia tirucalli* (L.) oil extracted from different stem diameters, in order to determine levels of FFAs in comparable with the international liquid biofuel standards, since the species is widely distributed in many parts of the country.

MATERIALS AND METHODS

The Study Areas

This study was conducted in three among the Tanzanian's agro-ecological zones namely, Kinzudi at Goba in Dar es Salaam Region, Ibihwa at Bahi in Dodoma Region and Iyela Ward in Mbeya City. These areas differ in terms of climatic and edaphic conditions that at the outset of the study were considered to cause different rates of physiological activities of *E. tirucalli* plants leading to differences in the qualities of the oil produced (URT, 1997; 2007a; 2007b; 2011).

Description of the Study Areas

Dodoma agro-ecological zone lies between latitudes 4^{o}S and 7^{o}S and longitudes 35^{o}E – 37^{o}E at an altitude of 1000-1500 metres above the sea level. This zone is semi – arid in nature with undulating plains with rocky hills, low scarps and well drained soils with low fertility. It has maximum and minimum annual temperatures of about 31^{o}C and 17^{o}C respectively, with annual average unreliable unimodal rainfall distribution of around 500-800mm (URT, 2007a; 2007b; URT, 2011; WHF, 2013). The Dar es Salaam agro-ecological zone lies between latitudes 6.45^{o}S and 7.25^{o}S, and longitudes 39^{o}E and 39.55^{o}E at an altitude of under 300 metres. This zone is tropical or warm and humid throughout the year having gently rolling uplands with moderately low fertility sand soils mixed with alluvial deposits in some parts. It has mean annual temperature of about 26^{o}C to 32^{o}C. Dodoma receives average annual bimodal rainfall of 750-1200mm (URT, 1997; 2007a; 2007b; 2011). Mbeya agro-ecological zone lies between latitudes 7^{o}S and 9^{o}S and Longitudes 32^{o}E and 35^{o}E. The zone has a tropical climate with mean annual unimodal and reliable rainfall of between 800-1400mm while the mean annual

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temperature is 21°C (URT, 1997; 2007b). Mbeya lies at an altitude of 1200-1500m having topography covered by undulating plains to dissected hills and mountains. It has moderately fertile clay and volcanic soils (URT, 1997; 2007b).

Preparation of Materials and Methods of Oil Extraction

Preparation and Gathering of Sample Materials

From each of the three study areas four bark strip samples each measuring 20cm wide by 20cm long were collected from *E. tirucalli* trees, at varying diameters of 20cm, 30cm, 40cm, 50cm, 60cm, 70cm and 80cm diameters at breast height (DBH) (Plate 1). The samples were kept separately in properly labeled plastic bags and transferred to the Chemical and Mining laboratory of the College of Engineering and Technology, University of Dar es Salaam where they were weighed to determine their fresh weight before being oven-dried to a constant weight at a temperature of 70°C. Using an electric milling machine the oven-dried samples were then pulverized into small-sized particles that could pass through a 2 mm-diameter mesh sieve and again weighed to determine their dry weight prior to extraction of the oil. Safety measures like proper cleaning of samples, were taken to avoid sample contamination in order to maximize the efficiency of extraction process.

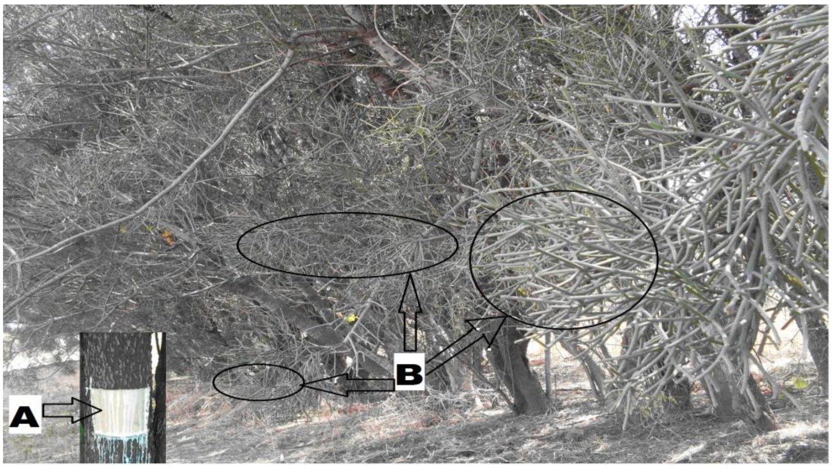

Plate 1 Wildly grown Euphorbia tirucalli trees showing locations of the 20cm x 20cm wildly grown stem bark strip cut from the tree at breast height (A) and Phylloclades (B).

Extraction and Separation of Oil Fractions from Stem Bark Samples of *E. tirucalli*

The extraction-partitioning scheme (Figure 1) was followed in the extraction of oil from the samples. During extraction, quadruplicate 20-g sub-samples from each of the finely ground stem bark collected from the three different study areas were extracted using 150mls of analytical grade acetone for eight hours in a Soxhlet apparatus according to the method adapted from Kalita and Saekia (2004).

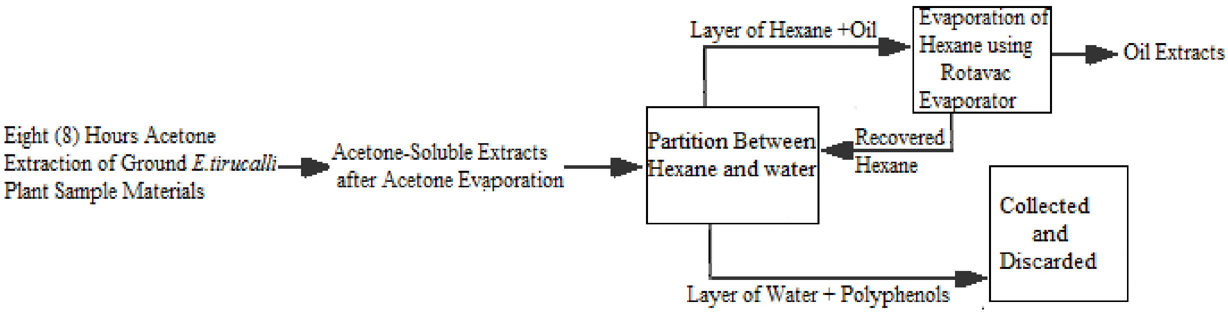

Figure 1. The extraction-Partitioning Scheme for Extraction of Oil adapted from Buchanan et al., (1978); Photi, (2005) and then Modified by the Researcher.

Consequently for each extract, acetone was evaporated out using a rotary evaporator at a temperature of 38°C to obtain a mixture of acetone-soluble extracts which were collected in a round bottomed flask. The obtained acetone-soluble extracts were each separately partitioned in a separating funnel using a mixture of analytical grade hexane and water. In that partitioning the oil fractions dissolved in hexane while the polar components (mainly polyphenols) dissolved in water. The oil fractions were then freed from hexane by evaporation in a rotary evaporator at a temperature of 68°C and the oil extracts that remained in the rotary evaporator were collected in previously weighed flasks and left to cool down for about 5 minutes before weighing the flasks again. Then, the weight of oil extracts was determined by subtracting the weight of the empty flask from the weight of the flask with oil and the obtained oil yield was stored in a refrigerator at 4°C until further used for the FFA experiments.

Determination of AVs and FFAs in Oil Samples

In order to assess the effects of FFAs on the quality of *Euphorbia tirucalli* (L.) oil from *E. tirucalli* plants for use as liquid biofuel, AVs and FFAs in oil samples were determined. This is because the fatty acid composition of plant oils has the major effect on fuel properties. Acid values of the extracted oils were determined by titration using the standard test method ASTM D 664 of the American Society for Testing and Materials ASTM D 6751standard methods (ASTM, 2003; 2007; 2009) and the Cd 3d-63 test method of the American Oil Chemists Society (AOCS, 1998). The AVs so obtained were used to calculate the %FFA as percent oleic acid by dividing the AVs by 1.99 as stated in the standard methods. Four oil samples obtained from stems with the same diameters were analyzed separately for the %FFA and the mean values obtained were recorded.

STATISTICAL ANALYSES

The data obtained in this study were subjected to statistical analysis whereby differences in the Mean AVs and FFAs of oils extracted from stem bark of *E. tirucalli* plants with different diameters and collected from different agro-ecological zones were analyzed using the analysis of variance (One-way ANOVA) ($P \leq 0.05$) with the aid of the Minitab software v.16 to test for statistical significance difference between them. Also comparison of the FFA values to verify their compliance with recommended ASTM D 6751 and EN 14214 standards was performed.

RESULTS AND DISCUSSION

AV is an important indicator of oil quality which is expressed as the amount of KOH (in milligrams) necessary to neutralize FFAs contained in 1 g of oil (Firestone, 1996). The results of mean AV and %FFAs of oils extracted from the wildly grown *E. tirucalli* stem bark samples collected from different agro-ecological zones are presented in Table 1 below. The results presented in Table 1 show that AVs of oils from different stem bark samples of *E. tirucalli* had a pick range of 9.11 mg KOH at 20cm to 9.38 mg KOH at 40cm (noted in Dodoma agro-ecological zone) compared to those from samples of other agro-ecological zones such as Mbeya, 7.18 mg KOH at 40cm to 7.32 mg KOH at 50cm and Dar es Salaam, 5.75 mg KOH at 20cm to 6.09 mg KOH at 60cm while the % FFAs in Dodoma agro-ecological zone was higher for about 4.57% at 20cm to 4.72% at 40cm than those from Mbeya, 3.61% at 40cm to 4.42% at 30cm and Dar es Salaam, 2.45% at 30cm to 3.00% at 80cm agro-ecological zones. The important observations deduced from Table 1 is that when %FFAs of *E. tirucalli* oils were compared with the American Standard for Testing and Materials (ASTM D 6751) and the European standard for liquid biofuel particularly biodiesel (EN 14214) which recommend for the %FFA levels < 0.020 % (ASTM, 2002), it was found that all the oils studied demonstrated poor quality in which %FFAs of oils from all stem bark samples in all agro-ecological zones fell outside the recommended standard Limits i.e., > 0.020 %.The results also revealed that, the obtained higher %FFA levels than recommended limits were due to higher AVs of oils (see for example, 9.11 mg KOH at 20cm to 9.38 mg KOH at 40cm recorded in Dodoma agro-ecological zone). Higher AVs in relation to % FFAs were also reported by Ankapong, (2010) who studied the physical and chemical properties of Palm Oil, Palm kernel Oil, Refined Soybean Oil, Unrefined Soybean Oil and *Jatropha curcas* oil. In that study, an AV of 10.10 was recorded for Palm oil which corresponded to high level of FFAs of 5.05% compared to 4.81%, 1.73%, 3.51% and 4.76% for Palm kernel oil, refined soybean oil, unrefined soybean oil and *Jatropha curcas* oil, respectively. Nevertheless, AVs were used to calculate %FFAs which were used to determine oil quality. Thus, description of the results was centered on % FFA.

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Table 1: Mean AVs and %FFA contents of oil extracted from wildly grown samples of E. tirucalli stem barks collected from different agro-ecological zones

Dodoma							
Stem Diameters (cm)	20	30	40	50	60	70	80
AVs of Oil from the stem bark (mg KOH)	9.11	9.23	9.38	9.36	9.32	9.35	9.29
% FFAs of Oil from the stem bark	4.57	4.64	4.72	4.7	4.68	4.7	4.67
Mbeya							
Stem Diameters (cm)	20	30	40	50	60	70	80
AVs of Oil from the stem bark (mg KOH)	7.28	7.3	7.18	7.32	7.22	7.31	7.29
%FFAs of Oil from stem bark	3.66	4.42	3.61	3.68	3.63	3.67	3.7
Dar es Salaam							
Stem Diameters (cm)	20	30	40	50	60	70	80
AVs of Oil from the stem bark (mg KOH)	5.75	5.87	5.91	5.85	6.09	5.93	5.97
%FFAs of Oil from the stem bark	2.89	2.45	2.95	2.94	3.06	2.98	3

One-way ANOVA results in Table 2 showed that despite poor in their quality, but differences in the quality of oils (%FFA) extracted from different stem diameters of the wildly grown *E. tirucalli* in different agro-ecological zones were statistically significantly higher ($p < 0.05$) at the 95% confidence limits.

Table 2: One-way ANOVA for differences in the quality of oil (%FFA) contents from stem bark samples of the wildly grown E. tirucalli in different agro-ecological zones at the 95% confidence limits

Source	DF	SS	MS	F	P
Factor	2	11.0016	5.5008	129.16	0.000
Error	18	0.7666	0.0426		
Total	20	11.7682			
Source = 0.2064, R-Sq = 93.49%, R -Sq (adj) = 92.76%					

Also based on Pooled Standard Deviation (StDev) - a weighted estimate of spread (variability) across samples using a weighted average, one-way ANOVA results presented in Table 3 revealed that, Dar es Salaam (0.2035 StDev), Dodoma (0.0505 StDev) and Mbeya (0.2895 StDev) agro-ecological zones did not share comparable standard deviations, and because the pooled standard deviation value (0.2064) obtained in this study deviated from standard deviations of all the studied agro-ecological zones. Thus, it is clear that differences in the quality of oils (%FFA) from different stem diameters of *E. tirucalli* in all agro-ecological zones were significantly higher at the 95% confidence levels.

Table 3. One-way ANOVA results for Individual 95% CIs for Mean Based on Pooled StDev

Level	N	Mean	StDev	----+---------+---------+---------+-----
Dar es Salaam	7	2.8957	0.2035	(-*--)
Dodoma	7	4.6686	0.0505	(--*--)
Mbeya	7	3.7671	0.2895	(--*--)
				----+---------+---------+---------+----- 3.00 3.60 4.20 4.80
Pooled StDev = 0.2064				

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From results in Table 3, the obtained higher %FFAs in different agro-ecological zones suggest that, in order for the *E. tirucalli* oil to be used to power engines reliably without any negative effects on engines such as formation of deposits at injection nozzles, pistonnes and valves it requires some treatment to reduce the %FFA content through conversion to the corresponding methyl or ethyl esters, thereby minimize the effects of FFAs in engines and other biodiesel-powered machines. Lower %FFAs in oils improves their quality and makes them meet the required specifications of liquid biofuel standards. Dorodo (2002) reported that transesterification would not occur if the oils have higher FFA content of more than 3%; but, Van Gerpan (2006) reported that the reaction can be catalyzed with an alkali catalyst if the vegetable oil has FFA content of up to about 5% instead of alcohol catalysis. However, it is known that oils with FFA content higher than 5% decrease the transesterification yield, through inhibiting the formation of methoxides by neutralization of part of the catalyst present and producing soaps within the reaction medium (Freedman *et al.*, 1984; van Gerpan, 2006). Soap formation would exacerbate the problem of phase separation at the stage of product recovery and as a result, lead to low yields of biodiesel. Furthermore, findings in Figure 1 shows that the quality of oils (%FFAs) has been increasing with increases in stems diameters considered in different agro-ecological zones. However, such increase was unsteady exhibiting an alternating upward and downward movement at low stem diameters between 20cm and 40cm, thereby increased continuously from 50cm towards 80cm diameters. The up and down features initially exhibited by the graph at low stem diameters might be possibly due to differences in biogenesis of oil bodies in tissues of small *E. tirucalli* plants which became unstable and coalesced rapidly, and then their accumulation peaks later as plant stem diameters increased in size (He and Wu, 2009). Also findings in Figure 2 indicated that the quality of oils (%FFAs) extracted from the wildly grown stem bark samples collected from *E. tirucalli* in Dodoma agro-ecological zone had significantly higher %FFA than those of the wildly grown stem bark samples in Mbeya, and Dar es Salaam agro-ecological zones in the same order (Figure 2). The differences in quality of oil (%FFA) from different stem diameters under different agro-ecological zones were associated to differences in soil characteristics and climatic conditions from which the samples had been collected.

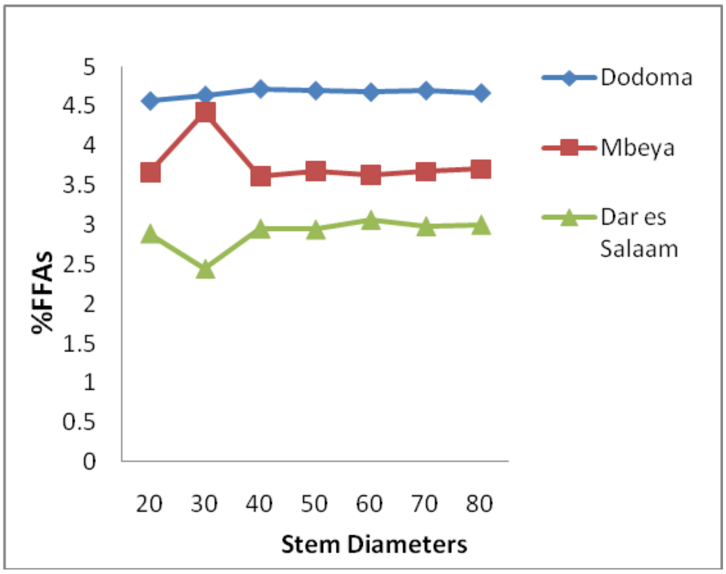

Figure 2. Differences in the mean %FFA contents of the oil from different stem diameters of E. tirucalli trees under different agro-ecological zones.

This was possibly because, there was higher nutrients uptake by *E. tirucalli* plants in Dodoma agro-ecological zone than Mbeya (intermediate) and Dar es Salaam (lowest) agro-ecological zones. Higher nutrient uptake increased physiological activities of the study plants which in turn increased biomass production and formation of oil bodies which lead to the increase in AVs and %FFAs of the oil. The increase in %FFAs reduced the quality of oil produced. Hence higher %FFAs obtained in *E. tirucalli* samples collected from stem diameters in all agro-ecological zones demonstrated low quality of oil. Furthermore, *E. tirucalli* has been described as a hard plant which can survive under a variety of edaphic and climatic conditions ranging from semi-arid to mesic

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climates (Calvin and Duke, 1980; 1983; Duke, 1983; FAO, 2012). This ability of *E. tirucalli* to survive in a variety of these conditions and particularly in the semi-arid conditions is due to its succulent nature which enables it to reserve water in its tissues for use during drought periods. As such it exists more or less independent of water supply from the soil during the peak of the dry season and its physiological activities proceeds as normal (Duke, 1983; FAO, 2012).

CONCLUSIONS

This research work was carried out in order to evaluate effects of FFAs on the quality of *Euphorbia tirucalli* (L.) oil extracted from different stem diameters in three different agro-ecological zones, i.e. Dodoma, Dar es Salaam and Mbeya. Thus, the conclusions that were drawn from this work are as follows: The study found that, *E. tirucalli* oil extracted from different stem diameters of *E. tirucalli* in different agro-ecological zones had higher % FFA above the levels recommended for liquid biofuel standards (FFA levels < 0.020 %) due to higher AV percentages. Thus, the study concluded that the direct use of *E. tirucalli* oil in stationary and moving machines including vehicle engines without pretreatment would be inappropriate because it would certainly lead to fouling of the fuel systems such as fuel injection systems in engines. In the present study, Dodoma agro-ecological zone was the most favourable area for the high FFAs of *E. tirucalli* oil followed by Mbeya and the least was the Dar es Salaam agro-ecological zone. This shows that *E. tirucalli* oils from Dodoma had the lowest quality than Mbeya and Dare s Salaam agro-ecological zones in that order. Also it was generally found that the differences in the quality of oils (%FFA) from different stem diameters of *E. tirucalli* in all agro-ecological zones were significantly higher at the 95% confidence intervals and found to increase from lower to higher stems diameters irrespective of agro-ecological zones.

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