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Oxidative Stability and Its Effects on the Quality of Oil Extracted from *E. tirucalli trees* in Different Agro-Ecological Zones of Tanzania

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Abstract:

Plant oils are prone to oxidation during long-term storage or through autoxidation. Oils may undergo conversion and degradation due to oxidation and polymerization reactions which can affect their quality. The present research studied oxidative stability and its effects on the quality of oil extracts from E. tirucalli trees found in Dodoma, Mbeya and Dar es Salaam agro-ecological zones in Tanzania. Oxidative stability (Mean induction time) was used as a parameter to evaluate the quality of oil. Oils were extracted from E. tirucalli stem bark samples with different diameters using Soxhlet extraction method. A 873 Biodiesel Rancimat Instrument was used to determine mean induction time of oils. Obtained oxidative stabilities were compared with the standards EN 14214-03 (6 hours) for biofuels suitable for running engines. Analysis of Variance (ANOVA) performed using Minitab software tested the differences in quality of oil. Results showed that oxidative stabilities which ranged from 3 to 5 hours from 3.12h + 0.23 - 5.08h + 0.23, 3.5h + 0.13 – 4.56h + 0.13 and 3.06h + 0.17 – 4.48h + 0.17 for Dodoma, Dar es Salaam and Mbeya respectively, were different from each other ($p < 0.05$). However, there were no clear trends of differences in oil oxidative stabilities among stem diameters and ecological zones. Also oxidative stabilities were lower than recommended biofuel standard EN 14214-03 (6h). The study concluded that the quality of oil was low and Dodoma offered higher quality of oil than Mbeya, and Dar es Salaam zones.

Keywords
E. tirucalli, oxidative stability, induction time, quality of oil, ecological zone, stem diameters, stem bark

1. INTRODUCTION

Plant oil is one among important sources of liquid biofuel which can be used as a heating or cooking fuel, and for use in internal combustion engines (diesel) (Meher *et al.*, 2006). Oil from plants can be extracted from existing food crops like rapeseed and sunflower seeds after being used for food preparation (waste vegetable oil) or even in their first use forms (Mariod *et al*., 2009). Non-food plants such as *Madhuca indica*, *Jatropha curcas* and *Pongamia pinnata* have also been used for oil extraction (Senthil *et al.*, 2003). Oil from these plants can also be used in biodiesel production under experimental conditions, which is a clean-burning diesel fuel produced from plant oils with their chemical structures made of fatty acid alkyl esters (Mariod, 2005; Meher *et al.*, 2006). Also, various plant parts such as roots, stems, leaves and flowers, have variable potentials for providing extracts and derivatives that are useful in producing oil for liquid biofuel purposes (Nielsen *et al*., 1977; Calvin, 1980; Mariod and Matthaus, 2004). Liquid biofuel is considered carbon neutral, as its biomass absorbs roughly the same amount of carbon dioxide during growth and when burnt (Mariod, 2005; Vaughn and Kenneth, 2016). Plant oils as fuel may give much lower toxic air emissions than fossil diesel, hence can be used in automobiles, home heating, and experimentally as a pure fuel itself (Mariod and Matthaus, 2004). The carbon chains of plant oils are 14 to 18 carbon atoms in length while those of fossil diesel fuel are 15 carbon atoms long which is quite close to the same size as plant oils (FARA, 2008). The composition of oils from plant parts has been reported by various authors (Nielsen *et al.*, 1977; Calvin, 1980; Ohyama *et al*., 1984) as components of the latex fractions of many plant species belonging to such diverse families as Asclepiadaceae, Moraceae and Euphorbiaceae (Calvin, 1978). Members of the family Euphorbiaceae have currently drawn great interest to researchers as biofuel crops because they produce white latex that is rich in fuel producing fractions such as oil, hydrocarbons and polyphenols (Saigo, 1983; Photi, 2005; Kalita, 2006). *Euphorbia tirucalli L.*, being a tree with C_3 and CAM metabolism in leaves and stems, grown on marginal, arid and semi-arid lands can be a good alternative source of oil for liquid biofuel. The plant belongs to genus *Euphorbia* within the family Euphorbiaceae (Priya and Rao, 2011). Its latex contains high amounts of sterols and triterpenes, and has been investigated for oil production. Nevertheless, prior to the use of plant oils as viable liquid biofuel either in their first use forms as stand-alone fuel or blended with other fuels, they must be analyzed to ensure that their quality satisfy among other quality parameters, the oxidative stability standards specified by the European Union (EU; EN 14214-03) which requires a minimum of six (6) hours and the United States (USA; ASTM D6751-08)

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that requires a minimum of three (3) hours for biofuels to resist oxidation (ASTM, 2003; DCG, 2009). The EN 14214-03 standard is adopted by many countries including Brazil, South Africa, India, Australian, United Kingdom and other 30 member states of the European Committee for Standardization (CEN) (European Commission, 2007); DCG, 2009). It describes a biofuel product (including oil) that can be used either as a stand-alone fuel or as a blending component in conventional based diesel fuel (European Commission, 2007). Thus, in the present study the EN 14214-03 standard was used to establish the oxidative stability (relative resistance of a liquid fuel to oxidation) of oils from *E. tirucalli* through its minimum induction time.

Furthermore, oxidative stability is an important issue for any oil due to its natural biodegradability which may occur not only during oil storage, but also during its production and use (Knothe and Dunn, 2003). Factors promoting oxidation are the presence of air, light, elevated temperatures, and the presence of extraneous materials such as the presence of metals (Knothe, 2010). Thus, oxidative stability is one among key aspects that determine the efficiency of oil quality and should be present at specific levels for a given liquid biofuel to be used in engines and other stationary diesel machines (Bozbas, 2005). Oxidative stability of liquid fuels particularly oil is of industrial concern, because, higher induction time guarantees that the oil can be used reliably under conditions of normal use, while lower induction time leads to accumulation of hydroperoxides or decomposition byproducts which eventually polymerize and form the insoluble sediments that are capable of plugging filters, fouling injectors and interfering with engine performance (Durrett, Benning and Ohlrogge, 2008; Karavalakis, Karonis and Stournas, 2009). The polymerization reaction can also lead to an increase in viscosity of liquid biofuels (Knothe, 2007). Long-term oxidative degradation of the oil can affect its quality such as increase in kinematic viscosity and acid values and decrease in the cetane number (Dunn, 2002; Monyem *et al.,* 2000; Lynch and Thompson, 1982). The oxidative stabilities of oils are also affected by many factors, including fatty acid composition, concentration and stability of antioxidants in the oil, and the presence of prooxidant compounds, such as free fatty acids (Knothe, 2007). Generally, plant oils as alternative sources of liquid biofuel can be susceptible to autoxidation. Their oxidation reactions start by the transformation of oils, especially those containing unsaturated double bonds to hydroperoxides which degrade their quality. The decrease in oxidative stability of oils results to increases in the degree of unsaturation hence affecting its quality (Hu, *et al.*, 2008; Greenwell, *et al.*, 2010; Ramos, *et al.*, 2009). Thus, in the current study, it was considered important to assess oxidative stability of oil content from *E. tirucalli* stem bark samples having different stem diameters such as 20cm, 30cm, 40cm, 50cm, 60cm, 70cm and 80cm gathered from Dar es Salaam, Dodoma and Mbeya as a means of assessing quality of differences in their oxidative stabilities.

2. MATERIALS AND METHODS

2.1 Description of the study areas

The study was conducted in three agro ecological zones of Tanzania such as central Dodoma, semi-arid land sub-zone, north, Dar es Salaam sub-zone and southern, Mbeya sub-zone. The study villages from three selected Agro ecological zones mentioned above were Ibihwa in Bahi District, Kinzudi village in Goba-Mbezi, and Iyela in Mbeya Urban (Figure 2.1). Selection of study areas having different agro-ecological zones was based on differences in altitudes, growth, precipitation and temperature patterns, as well as differences in edaphic and other physiographic features described by the National Adaptation Programme of Action (NAPA) (URT, 2007). These differences possibly offered differences in physiological activities which probably presented variations in quality of oils extracted from stem bark of *E. tirucalli* plants.

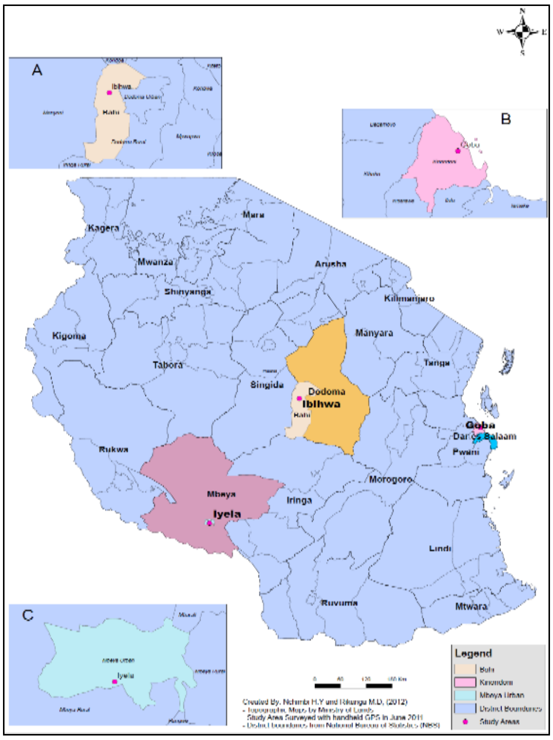

Figure 2.1: Map of Tanzania showing the locations of the study areas under different agro-ecological zones

2.1.1. Geographical locations, climate, soils and topography, altitude, rainfall and growing seasons of study areas

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2.1.1.1 Dodoma agro-ecological zone

The Dodoma agro-ecological zone is semi – arid in climate. This zone lies between latitudes 4ºS and 7ºS and longitudes 35ºE – 37ºE with an altitude of 1000-1500 metres above the sea level. Dodoma has undulating plains with rocky hills and low scarps. It has well drained soils with low fertility. Apart from having the average maximum and minimum annual temperatures of about 31ºC and 17ºC respectively, the region receives a year round average unreliable unimodal rainfall distribution of around 500-800mm (URT, 2007; URT, 2011; WHF, 2011). The growing season in Dodoma semi-arid sub-zone is during December and March.

2.1.1.2 Dar es Salaam agro-ecological zone

The Dar es Salaam agro-ecological zone is tropical or warm and humid climate throughout the year. It lies between latitudes 6.45ºS and 7.25ºS, and longitudes 39ºE and 39.55ºE with an altitude below 3000metres. Dodoma is gently rolling uplands with moderately low fertility sand soils and occupies soils mixed with alluvial deposits in some parts. Apart from having the mean annual temperature of about 26ºC which can rise to 32ºC during the hottest, the Region receives average annual bimodal rainfall of 750-1200mm (URT, 1997; 2007). The growing season in Dar es Salaam sub-zone is during October to December and March to June.

2.1.1.3 Mbeya agro-ecological zone

Mbeya agro-ecological zone experiences a tropical climate. It lies between latitudes 7ºS and 9ºS and between Longitudes 32ºE and 35ºE and has a mean annual unimodal and reliable rainfall of between 800-1400mm while the mean annual temperature is 21ºC (URT, 1997; 2007; Janssen *et al.*, 2005). Mbeya has an altitude of 1200-1500m while its topography is covered by undulating plains to dissected hills and mountains. The area has moderately fertile clay and volcanic soils. The growing season in Mbeya zone is during November to April (URT, 1997; 2007).

2.2. Preparation of materials and extraction techniques

2.2.1. Sample collection

Four bark strip samples each measuring 20cm wide by 20cm long were collected from *E. tirucalli* trees, each with 20cm, 30cm, 40cm, 50cm, 60cm, 70cm and 80cm diameters at breast height (DBH) from three study areas. The samples were separately kept in properly labeled plastic bags and transferred to the Chemical and Mining laboratory at 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 70ºC. Using an electric milling machine the oven-dried samples were then ground 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.

2.2.2. Extraction and separation of oil fractions from stem bark samples of *E. tirucalli*

The extraction-partitioning scheme (Figure 2.2) was employed in the extraction of oil from *E. tirucalli* samples. During extraction, quadruplicate 20-g sub-samples from each of the finely ground *E. tirucalli* stem bark samples collected from three different study areas were extracted using 150mls of analytical grade acetone for eight hours in a Soxhlet apparatus according to the method described by Kalita and Saekia (2004).

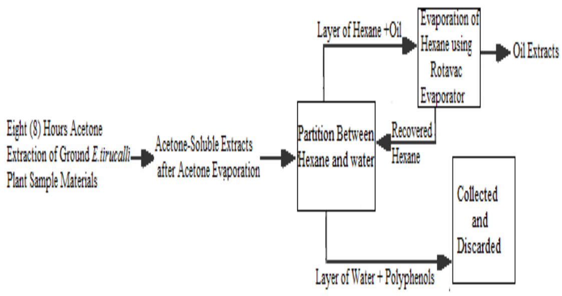

Figure 2.2: The extraction-partitioning design for extraction of oils from *E. tirucalli.* Adapted from Buchanan *et al.*, (1978); Photi, (2005) and then modified by researcher.

Then, for each extract acetone was evaporated out using a rotary evaporator at a temperature of 38ºC 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 68ºC 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 the flasks weighing again. Then, the weight of the oil extracts was determined by subtracting the weight of the empty flask from the weight of the flask with oil and

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the obtained oil yield was stored in refrigerator at 4°C for further use in oxidative stability assessments.

2.2.3. Determination of oxidative stability

Oxidative stability measurements of oils extracted from stem bark samples of *E. tirucalli* were determined in quadruplicate by using the accelerated oxidation test EN 14112 described in the standard methods EN 14214 – 03 of the European Committee for Standardization (AOCS, 1998; DCG, 2009). The instrument used was a 873 Biodiesel Rancimat (Knothe, 2009) available in the laboratory at the University of Dar es salaam (Chemical and Mining; College of Engineering and Technology). As specified in the method, oxidation was induced in 5mls oil samples under a continuous heating block temperature of 110°C and constant air flow of 10 L/h. In this case four oil samples from stems with the same stem diameters (diameters) from different study areas were analyzed separately and oxidative stability values were recorded based on induction time.

3.0 STATISTICAL ANALYSES

The obtained oxidative stability data were subjected to statistical analysis with the aid of the Minitab software v.16 to test whether they were statistically significantly different. Their differences were analyzed using the analysis of variance (One-way ANOVA) ($P \leq 0.05$) from the same software. Also, the induction times (oxidative stabilities) of oils so obtained were compared to European Biodiesel Standard (EN 14214-03) for quality of biofuel suitable for running engines and other stationary machines. This standard requires 6 hours induction time.

4. RESULTS AND DISCUSSION

4.1. Effects of oxidative stability on the quality of oil extracts of *E .tirucalli* from different agro-ecological zones

Oxidative stability is an important parameter for evaluating the quality of oils because it gives a good estimation of their susceptibility to oxidative deterioration which is the main cause of their alteration and decline in quality (Mariod, 2005). The results presented in Table 4.1 show that oxidative stabilities of oils from different stem bark samples of *E. tirucalli* had a pick range of 3 to 5 hours (noted in Dodoma agro-ecological zone) considered in all oil samples from different agro-ecological zones such as 3.12h ± 0.23 - 5.08h ± 0.23 for samples collected from the Dodoma; 3.5h ± 0.13 – 4.56h ± 0.13 for samples collected from the Mbeya and 3.06h ± 0.17 – 4.48h ± 0.17 for samples collected from the Dar es Salaam agro-ecological zones.

Table 4.1 Oxidative Stability of Oil (Mean induction time ± Standard error) Extracts of *E. tirucalli* Stem bark samples from Different Agro-Ecological zones.

Dodoma							
Stem's Diameters	20	30	40	50	60	70	80
Oxidative Stability of Oil from the stem bark (h)	4 ± 0.23	3.7 ± 0.23	3.74 ± 0.23	3.12 ± 0.23	4.16 ± 0.23	3.74 ± 0.23	5.08 ± 0.23
Mbeya							
Stem's Diameters	20	30	40	50	60	70	80
Oxidative Stability of Oil from stem bark (h)	4.12 ± 0.13	3.5 ± 0.13	4.06 ± 0.13	4.1 ± 0.13	3.94 ± 0.13	4.56 ± 0.13	3.75 ± 0.13
Dar es Salaam							
Stem's Diameters	20	30	40	50	60	70	80
Oxidative Stability of Oil from the stem bark (h)	3.06 ± 0.17	4.48 ± 0.17	3.52 ± 0.17	3.69 ± 0.17	3.45 ± 0.17	3.61 ± 0.17	3.99 ± 0.17

Thus, the obtained oxidative stabilities (mean induction times) were lower than recommended EU standard (6 hours) as presented in Table 4.1. Also see Figure 4.1 and 4.2 as Examples.

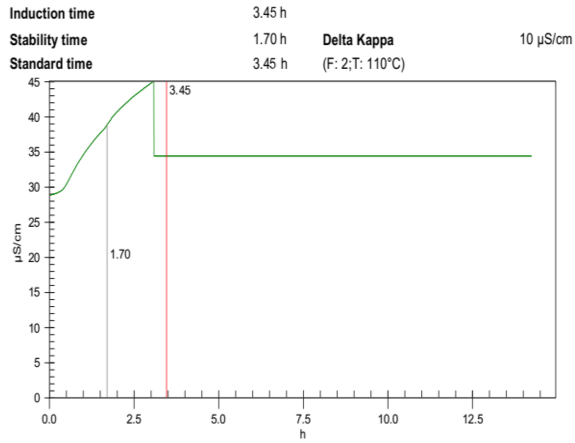

Figure 4.1 Oxidative stability of one sample oil from *E. tirucalli* extracted from 60cm stem diameters in Mbeya agro-ecological zone showing 3.45hours induction time.

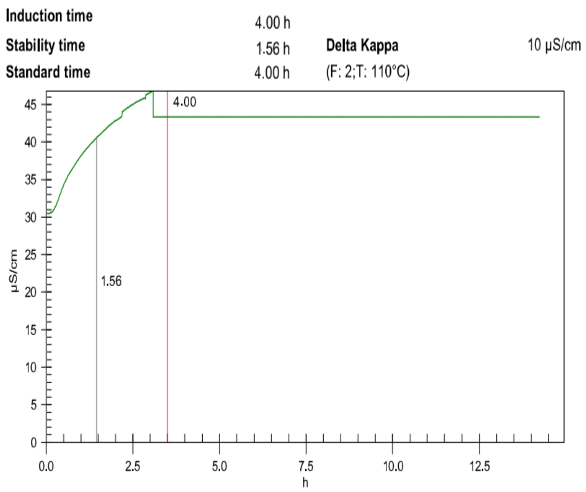

Figure 4.2 Oxidative stability of one sample oil from *E. tirucalli* extracted from 20cm stem diameters in Dodoma agro-ecological zone showing 4.00 hours induction time

Since, the obtained lower oxidative stability means that the quality of oils extracted from the stem bark samples collected from all the studied agro-ecological zones are poor and not qualifying the six (6) hours recommended liquid biofuel quality standards set by EN 14214-03 (6h). Hence, the quality of oils from different stem diameters of *E. tirucalli* collected from different agro ecological zones does not qualify for being used as liquid biofuel for running engines and other domestic stationary machines. These results differed from those earlier reported by Anwar and Bhange (2007) who obtained significantly higher oxidation stability in different plant parts from extracts of *Moringa oleifera* as a non-conventional source of biofuel. Also, different results were obtained by Mariod *et al*,. (2009) who investigated the oxidative stability of Marula oil in Sudan and reported significantly higher oxidative stability. Again, the *Sclerocarya birrea* kernel oil was studied with regard to oxidative stability among other parameters, and a high oxidative stability was reported (Mariod and Matthaus, 2004). Furthermore, the low oxidative stability obtained from *E. tirucalli* oil may probably be due to the effects of higher degree of unsaturation and low percentage of monosaturated fatty acids in addition to other minor bioactive components such as sterols and phenolics (Hidalgo and Zamora, 2005; Mariod, 2005). Given that, some authors have differently reported that one of the most important characteristics affecting oxidative stability is the degree of unsaturation of the oil (Hu, *et al.*, 2008; Greenwell, *et al.*, 2010; Ramos, *et al.*, 2009). Thus, it is probable that the oxidative stabilities of *E. tirucalli* oils have been decreasing due to increases in the degree of unsaturation and decrease in the percentage of monosaturated fatty acids. Furthermore, the low oxidation stabilities of the oil extracted from *E. tirucalli* indicates probability of low presence of natural antioxidants in the extracts of this species which reduced oxidation stability of oil. However, the amount, types and stability of these antioxidant substances in *E. tirucalli* plant parts were not determined due to limited capacity. Similar findings were reported by different studies which pointed out that antioxidant activities and oxidation stability of oils may be related to their contents of fatty acids which reduced oil deterioration. For example, Meriod *et al.*, (2005) and Baldioli, *et al.*, (1996) reported low oxidation stability and a remarkable antioxidant activity in Sunflower oil due to similar reasons. Again, the antioxidant activities of 3,4-dihydroxyphenylethanoland and phenyl acids (caffeic acid, *p*-coumaric acid, ferulic acid, syringic acid, and vanillic acid) have been reportedly high in virgin olive oil (Mariod, *et al.*, 2006; Mariod, *et al*,. (2008). On the other hand, results demonstrated that, regardless of the poor quality of oils obtained from different stem diameters of *E. tirucalli* bark samples in different agro-ecological zones. There were differences in qualities (oxidative stabilities) of oil (Table 4.1) among stem diameters between different agro-ecological zones used in the present study. One-way ANOVA (Table 4.2) results in Table 4.2 reveals that, these differences in the oxidative stabilities of oil extracted from the wildly grown *E. tirucalli* among different stem diameters and between different agro-ecological zones were significant at the $p < 0.05$ level. Also, despite their differences, but results (Table 4.1) show that there were no clear cut trends in the increasing qualities of oil from lower to higher stem diameters within and across agro-ecological zones i.e., oils from trees having larger stem diameters did not produce higher oxidative stabilities than oils from trees with small stem diameters.

Table 4.2 One-way ANOVA for the oxidative stabilities (Mean Induction Time) of oil from the stem bark at the 95% confidence limits.

One-way ANOVA: Oil from the Stem bark with Different Stem Diameters between Agro-Ecological Zones							
Source	DF	SS	MS	F	P		
Factor	1	22339	22339	106.32	0.000		
Error	40	8404	210				
Total	41	30744					
Source = 14.50, R-Sq = 72.66%, R –Sq (adj) = 71.98%							
Level					N	Mean	SE Mean
Mean Induction Time of Oil from the Stem Bark					21	3.87	0.103
Stem Diameters					21	50	4.47

Similarly, the data presented above show that despite lower oxidative stability values obtained from oil of *E.*

tirucalli stem bark samples in different agro-ecological zones, the oxidative stabilities of oil from Dodoma agro-ecological zone were almost close to the recommended EU standards (6h) (i.e., between 3 – 5 hours) higher than those of the oil extracted from the wildly grown stem bark samples of the same species collected from Mbeya (between 3.75 – 4.12 hours) and Dar es Salaam (between 3.06 – 4.48 hours) agro-ecological zones. Higher oil qualities in Dodoma than other agro-ecological zones can be attributed by the fact that *E. tirucalli* plant has been described as a hard plant which can survive under a variety of climatic regimes ranging from semi-arid to mesic climatic conditions (Calvin, 1980; Duke, 1983). This ability of *E. tirucalli* to survive in a variety of climatic 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. Also, the use of phylloclades instead of leaves for photosynthesis gives an extra survival advantage of *E. tirucalli* to semi- arid conditions, since the plant can be able to combine both CO_2-fixation in the leaves with Crassulacean Acid Metabolism (CAM) in their green stems. The stems can open their stomata and absorb carbon dioxide at night when it is cool thereby minimizing water loss through the stomata and increasing water use efficiency. According to Van Damme, (1989; 2001) this mechanism offers an additional ability of *E. tirucalli* plants to increase their metabolic rates and thereby maximize their yields particularly in semi-arid conditions. Moreover, the obtained results for differences in the quality of oil from stem bark samples *of E. tirucalli* can be supported by the influence of variations in environmental conditions in which the samples were collected. Since the samples were collected from different agro-ecological zones which are having disparities in environmental conditions such as topographical locations, climate, soils (nutrients) and altitude. Therefore, these conditions offer differences in terms of physiological activities of the study plant which led to differences in qualities of their oil produced. Finally, there is a relationship between degree of unsaturation and viscosity of the oil. According to Mateos *et al.*, (2005) oils containing fatty acids of low molecular weight are slightly less viscous than oils of an equivalent degree of unsaturation containing only high-molecular-weight acids. This means that the viscosity of the oil decreased slightly with increases in degree of unsaturation. Thus, it can possibly be said that the viscosity of the *E. tirucalli* oil also decreased slightly with increases in degree of unsaturation. Likewise, Mariod and Matthaus, (2004) and Mariod *et al*., (2009) extracted oil from *Sclerocarya birrea* and reported that the oil content from the species was less viscous compared with sesame, groundnut and sunflower oils due to similar reasons.

5. CONCLUSION

The present research work was carried out to evaluate the effects of oxidative stability on the quality of oil extracted from the wildly grown *E. tirucalli* trees, through differences in induction times of oils from stems with different diameters such as 20cm, 30cm, 40cm, 50cm, 60cm, 70cm and 80cm as a potential sources of liquid biofuel 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 oxidative stability indices (Mean Induction Time) of oils from the stem bark of *E. tirucalli* trees with different stem diameters did not qualify the recommended minimum standards of 6 hours according to EN 14214. This implies poor quality of extracted oils, and indicates that the direct use of *E. tirucalli* oil in diesel engines and other domestic machines without pretreatment would be impossible because it would certainly lead to the fouling of the fuel injection systems in engines. Findings also concludes that, the oxidative stabilities of oil from the wildly grown stem bark samples with different diameters in Dodoma agro-ecological zones were slightly significantly higher than that of Mbeya and Dar es Salaam agro-ecological zones. These results confirm that, despite poor oil quality, but the quality of oils from the stem bark of *E. tirucalli* plants with different diameters from Dodoma agro-ecological zone are fairly higher than those of the plants from Mbeya, and Dar es Salaam agro-ecological zones. Hence, for mass production of *E. tirucalli* oil as regards to quality studies, Dodoma offers better opportunities in terms of the qualities of oil when compared with the Mbeya and Dar es Salaam agro-ecological zones. Also the study concludes that, there were no clear cut trends in the quality of oil from *E. tirucalli* among stem diameters across different agro - ecological zones.

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