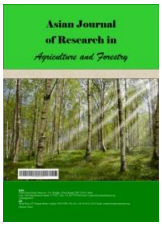

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Effect of Clonal Variation on Oil Content and Oil Properties of *Calophyllum inophyllum* L: A Multi Purpose Tree

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Calophyllum inophyllum is a potential source of non-edible seed oil. Calophyllum oil is used as a biofuel and also for medicinal purposes. At the Institute of Forest Genetics and Tree Breeding, Coimbatore carried out a study in 2019, on five different clones of *C. inophyllum* to investigate the effect of clonal variabilty on oil yield and oil physico-chemical properties for use as a biofuel. Oil yield significantly varied among the five clones and ranged from 48.6% to 55.8%. Among the physical properties, except Kinematic Viscosity, Specific Gravity and Refractive Index, the properties such as Flash point, Fire point, Pour point and Cloud point did not vary significantly. However, all the chemical properties studied, such as Acid value, Saponification value, Iodine value

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and Peroxide value showed apparent variation. From the study it could be derived that clone 15 is more suitable as tree crop for biofuel purpose and the variations among the clones indicate prevalence of scope for tree improvement of *C. inophyllum* for specific use.

Keywords: Calophyllum; oil yield; viscosity; biofuel; acid value; specific gravity; peroxide value.

1. INTRODUCTION

Calophyllum inophyllum, is a non –edible oilseed evergreen tree that belongs to the family *Clusiaceae.* In Tamil it is known as *Punnai, Punna in Malayalam, Sara Honne in Kannada* and commonly referred as "Alexandrian Laurel" in English. This tree can grow 8 to 20 metres tall with a broad spreading crown of irregular branches which exudes white latex when bruised [1]. Flowering occurs year round, but usually two distinct flowering periods are observed, in late spring and in late autumn. The fruit is a purplish black globoid-to-ovoid drupe when mature with a single seed. The fruit epicarp, which turns yellow and then brown and wrinkled when ripe, covers the shell, a corky inner layer, and a single seed kernel.

Calophyllum inophyllum is native to India, many Southeast Asian countries, Sri Lanka, East Africa, Madagascar, Vanuatu, Fiji and Samoa. Within India, it is distributed in the coastal and riverine belts of Tamil Nadu, Kerala, Karnataka, Andhra Pradesh, Orissa, Maharashtra and the And a mans. Due to its wide distribution, *Calophyllum inophyllum* populations are expected to exhibit wide variability which can be utilized for tree improvement. The morphological variations in seed characteristics among the natural population are useful in selection programme for genetic improvement of forest species [2,3].

Its kernels can yield a green tinted non edible oil to the range of 40-73%. Calophyllum oil has both medicinal importance and biofuel potential. Methods for transesterification of Calophyllum oil to biodiesel are available [4-7]. However it has been reported that the non- transesterified calophyllum oil can also be used directly as Pure Plant Oil (PPO) to run agricultural pumpsets [8]. Calophyllum oil or Tamanu oil is traditionally known for its rich medicinal value [9]. The Calophyllum oil possesses healing potential and accelerates new tissue development for healthy skin [10]. The oil is widely used in to relieve neuralgia, rheumatism, sciatica to cure wounds, burns, insect bites and stings, acne and acne scars, psoriasis, diabetic sores, anal fissures, sunburn, dry or scaly skin, blisters, eczema, and herpes sores, and to reduce foot and body odor and possesses anti-cancer properties [11,12]. Due to its medicinal value it fetches high price in international market and can be employed in soap- making. Presently in India, the Calophyllum oil is traded for medicinal use at a cost of Rs.400 to 950/L. The timber of this species referred as 'Bintagor', is of superior quality and is popularly traded in South-East Asian countries. Calophyllum wood has demand for carving, furniture making, boat building, and flooring. It is an excellent urban forestry species for shade along roads. The tree is a good host for rearing honey bees for apiculture. Due to the resistance of the species to salt sprays it acts as a superior bioshield along the coasts. It grows well in riverine areas and stabilizes loose soil. It adapts well in sandy, clayey, calcerous soils and can be used to green such underutilized lands. Thus *C. inophyllum* has been found to be a promising multipurpose tree suitable for Southern India. It is reported that the typical yield of an adult tree is around 5 kg of cold pressed oil is produced for every 100 kg of fruit [13]. In the present study, the variability expressed by different *C. inophyllum* clones in its oil content and oil physico-chemical properties was studied for the purpose of tree improvement and better utilization.

2. MATERIALS AND METHODS

2.1 Fruit Collection and Processing

Mature fruits of *Calophyllum inophyllum* at yellow green stage were collected from a six year old clonal trial established by IFGTB at Salem (11.76◦N; 78.16◦E; 278 msl) during December 2019. Seeds of five different clones C-15 [Kollam; 8.57◦N; 76.31◦E; 9 msl], C-26[Cuddalore; 11.30◦N; 79.42◦E; 11 msl], C-33[Kasaragod; 12.38◦N; 74.55◦E; 7 msl], C-88 [Nagapattinam; 10.33◦N; 79.50◦E; 7 msl], and C-104[Coimbatore; 10.28◦N; 76.56◦E; 334 msl] of *Calophyllum inophyllum* were selected for the study. The fruits were cut open to release the nuts and stored at room temperature (28 ± 2°C ; RH 58±2%) for a month. Later the nuts were broken to extract the kernel and the initial

moisture content of the kernel was estimated. The kernels were shade dried at room temperature to attain 5% moisture content. At that stage the dry kernels will turn golden brown in colour with an oily luster.

2.2 Seed Moisture Content

The kernels were tested for moisture content on fresh weight basis by oven dry method [14]. About 5 g of seeds were taken in duplicates and determined the exact wet weights. The seeds were dried in a hot air oven at 103°C for 17 hours. The seeds were then cooled in desiccators and the dry weight was found. The moisture content was calculated using the following formula,

$$\text{Moisture content (\%)} = \frac{(\text{Initial weight} - \text{Dry weight})\ g}{\text{Initial weight (g)}} \times 100$$

2.3 Oil Extraction

Oil extractions were performed using 10 g of Calophyllum seed kernel powder per sample. Petroleum ether (boiling point: 40 to 60°C) was used as solvent for extraction as prescribed by AOAC 920.39C method [15]. It was refluxed for 2 hours to extract the oil from the kernel in a Soxtherm (Pelican Instruments, India). The oil content was expressed in percentage.

$$\text{Oil content \%} = \frac{\text{Weight of oil obtained after extraction}}{\text{Weight of the seed sample}} \times 100$$

2.4 Physical Properties Oil

2.4.1 Specific gravity

Empty weight of the bottle was noted (A1 g).Then noted the weight of bottle with water as (A2 g). Divided A1/A2 g to get X. Likewise noted the empty weight of bottle as (B 1 g) and weight with oil as (B 2 g). Divided B1/B2 g to get Y. The specific gravity was calculated by Y/X g.

2.4.2 Viscosity

Using DV-E Brookfield Viscometer the viscosity of oil was analysed according to ASTM D2983-09 standard [16]. Added 7ml of oil into the sample tube, set 30rpm, and spindle number selected was S18 which was immersed completely into the oil. The reading displayed indicated the kinematic viscosity unit in Centipoise or milli pascal per second.

2.4.3 Refractive index

Refractive index of oil was determined using the Abbe refractometer. The refractometer was first standardized to 1.3333 using distilled water at a temperature of 30°C. This water was cleaned off with tissue paper and replaced with about 0.5 g of oil sample. The dark and light regions of the refractometer were adjusted to meet at an intercept of a crossbar before the readings were taken [17].

2.4.4 Fire point and flash point

The fire point of a fuel is the temperature at which it will continue to burn after ignition for at least 5 seconds. At the flash point, a temperature lower than that of fire point, the substance will ignite but vapor might not be produced at a rate to sustain the fire. The respective temperatures were determined using Pensky Marten apparatus [18].

2.4.5 Cloud point and pour point

The cloud point is defined as the temperature of a liquid specimen when the smallest observable cluster of wax crystals first appears upon cooling under prescribed conditions. The pour point describes a procedure for testing the fluidity of a fuel at a specified temperature. Cloud point and Pour point were measured for the Calophyllum oil using the Cloud point and Pour point apparatus (Culture Instruments, India) as per ASTM D2500 and ASTM D93 respectively standards.

2.5 Chemical Properties of Oil

2.5.1 Acid value

The oil samples were subjected to chemical characterization for acid value [19,15] an important indicator of vegetable oil quality [20]. Acid value is expressed as the amount of potassium hydroxide (in milligrams) necessary to neutralize free fatty acids contained in 1 g of oil.

Acid value was determined for each oil sample by dissolving 0.20g of each oil in 2.5 ml of 1:1 v/v ethanol: diethyl ether solvent and titrating with 0.1N sodium hydroxide (NaOH) while swirling using phenolphthalein as indicator. Calculation is as follows,

$$\text{Acid value} = \frac{\{56.1\times N\times V\}}{W}$$

Where,

N = Normality of NaOH used
V = Volume (ml) of NaOH used
W = Weight of sample used

2.5.2 Saponification value

Saponification value which is a measure of fatty acid chain length in oils was determined [21,15] and expressed in milligrams of potassium hydroxide absorbed per gram of oil. One gram of oil was dissolved in 12.5 ml of 0.5% ethanolic potassium hydroxide and the mixture refluxed for 30 minutes. 1 ml of phenolphthalein indicator was added and the hot soap solution titrated with 0.5N hydrochloric acid. A blank determination was also carried out under the same condition and saponification value determined using the following equation,

$$\text{Saponification value} = \frac{56.1N\ (V_1 - V_2)}{W}$$

Where,

N = Normality of Hydrochloric acid used
V_1 = Volume of Hydrochloric used in test
V_2= Volume of Hydrochloric acid used in blank
W = Weight of oil used (1g)

2.5.3 Iodine value

The iodine value is a measure of the degree of unsaturation in an oil. Iodine value or number is defined as the g of iodine absorbed by 100g of the oil. The first step is to standardize the 0.1 N sodium thiosulphate by Taking 0.2835g of iodine ,0.6 g of KI in clean conical flask with 25ml of water. Titrate against 0.1N sodium thiosulphate. The end point indicates the appearance of straw yellow colour. Next titration is followed with 0.25g oil, dissolved in 10ml of chloroform and 25 ml of hames iodine, allow it to stand in the dark for 30 min by occasional shaking. Add 10 ml of 15% KI .Quickly titrated with std 0.1N thiosulphate to get pale yellow coloured solution. Then add 0.2 ml of freshly prepared starch solution. Titrate against sodium thiosulphate. End point is disaapearence of blue colour. Blank titration is carried out without oil [21,15]. The Iodine value was calculated by the formula,

Iodine value= (B - S) × N × 12.69/sample in gm

Where,

B= ml thiosulphate for blank
S= ml thiosulphate for sample
N= normality of thiosulphate solution

2.5.4 Peroxide value

Peroxide value was determined [19,15] and expressed in milli Eq / Kg of oil is an index of fatty acid oxidation.One g of each oil sample was weighed into a 200 ml conical flask then 25 ml of 2:1 v/v glacial acetic acid chloroform solvent was added 1 ml of saturated potassium iodine was then added and mixture left in the dark for 1 minute. Next, 30 ml of water was added and the mixture titrated with 0.02N thiosulphate solution using 5 ml starch as indicator. A blank determination was similarly carried out. Peroxide Value was calculated from the equation,

$$\text{Peroxide value} = \frac{\{100\ (V_1 - V_2)\ mg/kg\}}{W}$$

Where,

W = weight of sample
V_1 = volume (ml) of thiosulphate used in test
V_2 = volume (ml) of thiosulphate used in blank

2.6 Statistical Analysis

The experiments were carried out in Completely Randomized Design with four replications each. One-way ANOVA was used to test the efficacy of the treatments, and means that exhibited significant differences were compared at 5% level of confidence (α = 0.05) using GENSTAT 5.0 software.

3. RESULTS AND DISCUSSION

The oil content in each of the five clones were quantified and the results obtained are furnished in Table 1. The physical properties namely Kinematic Viscosity, Specific gravity, Refractive index, Flash point, Fire point, Pour point and Cloud point of five different clones of *Calophyllum inophyllum* seed oil have also been tabulated in Table 1.

In India, studies on distribution, genetic variation, selection and germplasm bank establishment can be taken up to initiate genetic improvement of *C. inophyllum* for attaining higher productivity [22]. Clonal variations in *C. inophyllum*, with respect to physiological and biochemical traits in this species across locations indicated that specific clones of *C. inophyllum* can with stand moderately harsh climatic conditions and can be

deployed for raising plantations with high productivity [6]. Pavithra et al. [23] reported that variability in fruit and seed have relationship with the genetic potential of a genotype. In *C. inophyllum*, based on the growing condition, significant variations were recorded in seeds collected from Maharashtra sources which were bigger and bolder than those Karnataka seed source [24]. Variations in physico-chemical properties of seed oil have been reported in *Jatropha curcas* [25].

In this study, the Calophyllum oil content significantly varied among the five different clones ranging from 48.6% to 55.8%. According to Chavan et al. [26] the oil percentage of Calophyllum was 55-75%. The Calophyllum oil content varies significantly between trees, seasons and the locations. These variations can range from 20% to 70% [27]. The variations in oil content in *C. inophyllum* were highly influenced by the provenance contribution (99.8%) [28]. Similar reports were obtained for oil from *Pongamia pinnata* [3].

The oil physical properties such as flash point, fire point, pour point and cloud point did not vary significantly among the five different clones of *Calophyllum inophyllum.* However the viscosity, specific gravity and refractive index showed significant variation. Viscosity is the most important property that affects the fluidity of the fuel. Viscosity is a measure of an oil's resistance to flow. It decreases (thins) with increasing temperature and increases (or thickens) with decreased temperature. Another factor in the measurement of viscosity is the ability of an oil to resist shearing during the hydrodynamic lubrication function. The kinematic viscosity of oil from the five clones C-15,C-26,C-33,C-88 and C-104 at 40°C were found to be 40.8, 40.5, 41, 40.4 and 40.9 centistokes (cST) respectively. The highest viscosity was recorded in C-33 and the lowest in C-88 . Venkanna and Reddy [29] determined the kinematic viscosity of *Calophyllum inophyllum* oil at 40^0C as 32.48. Elangovan et al. [30], reported the viscosity as 38.47 at 40^0C for *C.inophyllum* oil. Viscosity of *C. inophyllum* oil lies inside the range of literature value, which is 28.27-56.7 cP [31,26,32]. Wulandari et al. [33], reported in Calophyllum oil, the properties such as viscosity at 40^oC, flash point and pour point to be 29.995 cts, 112^oC and 89^oC respectively.

The highest specific gravity of *C.inophyllum* oil recorded in this study was found to be C-104(0.965) followed by C-88 (0.956), C-26 (0.954), C-33 (0.947) and C-15 (0.94467). Arumugam and Ponnusami [4] reported the specific gravity of *Calophyllum inophyllum* seed oil to be in the range of 0.91 – 0.96. Suchith kumar and Dahananjaya [34] determined the specific gravity of *C.inophyllum* oil as 0.81.

The higher refractive index of the oil was found to be both C-26 and C-104 (1.46767). C-33 shows the refractive index of 1.46700. The least refractive index were found to be C-15 and C-88 (1.46633) respectively. Atabani et al. [35] determined the refractive index of *C. inophyllum* oil as 1.478. Phila Raharivelomanana et al. [36] reported the refractive index of *C.inophyllum* oil was found to be 1 .474.

In the present study the mean flash point, fire point, pour point and cloud point of Calophyllum oil was recorded as 224.20^oC, 246.67^oC, 4.13^oC and -4.07^oC respectively, but clonal variation in these properties were insignificant. The flash point is the temperature at which fuel vapor given off momentary flash when an external flame is introduced under specified test conditions. It is an important parameter for safe storage and handling of fuel. Elangovan et al. [30] reported the flash point of *C.inophyllum* oil were found to be 224^oC.Sharanabasappa et al.[37] determined the flash point of *Calophyllum inophyllum*oil were found to be 220^0C. The flash point of Pongamia oil was about 240°C [38,39,40]. Elangovan et al. [30] reported the fire point of *C.inophyllum* oil to be 253°C. Sharanabasappa et al. [37] determined the fire point of *Calophyllum inophyllum* oil as 230°C. Arumugam and Ponnusamy [4] reported the pour point of *C. inophyllum* oil were found to be 8°C. Ganesh and Chandrasekaran [41] determined the pour point of *C. inophyllum* oil to be 4.3°C. The cloud point means the temperature at which a sample of the fuel starts to become cloudy when the fuel is cooled in prescribed conditions. Arumugam and Ponnusamy [4] reported the cloud point of *C. inophyllum* oil were found to be -2.5°C. The cloud point of vegetable oil-Pongamia oil was about 8^oC [38,42,43].

Oil quality is important when producing oil for direct use as a fuel. In general, it is necessary to ensure low contamination of the oil, low acid value and high oxidation stability [44]. The chemical properties of oil namely Acid value, Saponification value, Iodine value, Peroxide value were studied for five different clones and tabulated in Table 2.

Table 1. Oil Content and physical properties of *Calophyllum inophyllum* seed oil

Clone No.	Oil Content %	Kinematic Viscosity at 40°C (cST)	Specific gravity	Refractive Index	Flash point (°C)	Fire point (°C)	Pour point (°C)	Cloud point (°C)
15	55.8[a]	40.8[a]	0.94467[c]	1.46633[b]	223.67	245.67	4.33	-4.00
26	48.6[d]	40.5[b]	0.95400[b]	1.46767[a]	224.00	247.33	4.00	-4.00
33	53.8 [b]	41.0[a]	0.94700[c]	1.46700[b]	224.00	246.33	4.00	-5.00
88	48.8[c]	40.4[b]	0.95600[b]	1.46633[b]	224.33	247.00	4.67	-3.67
104	49.5[c]	40.9[a]	0.96500[a]	1.46767[a]	225.00	247.00	3.67	-3.67
Mean	51.3	40.7	0.95333	1.46700	224.20	246.67	4.13	-4.07
S .e .d	0.326	0.1751	0.001687	0.000919	0.699	0.632	0.632	0.596
L.s .d	0.694	0.3733	0.003758	0.002048	1.558	1.409	1.409	1.329
	S	S	S	S	NS	NS	NS	NS

S- Significant at 5% level of confidence; NS- Not significant; S.e.d-Standard error of deviation; L.s.d-Least significant difference; Values with similar superscripts do not differ significantly at 5% level of significance

Table 2. Chemical properties of *Calophyllum inophyllum* seed oil

Clone no.	Acid value (mg KOH/g oil)	Saponification value (mg KOH/g oil)	Iodine value (g I_2/100 g oil)	Peroxide value (milli Eq / Kg oil)
C-15	16.70[b]	195.67[c]	85.03[b]	4.67[c]
C-26	16.53[c]	197.00[b]	85.53[a]	4.70[b]
C-33	17.30[a]	197.67[a]	84.57[b]	4.57[d]
C-88	16.53[c]	196.33[c]	81.93[c]	5.27[a]
C-104	16.70[b]	196.67[b]	85.57[a]	4.67[c]
Mean	16.75	196.67	84.53	4.77
S.e.d	0.0869	0.377	0.377	0.0558
L.s.d	0.1937	0.840	0.840	0.1243
	S	S	S	S

S- Significant at 5% level of confidence; NS- Not significant; S.e.d-Standard error of deviation; L.s.d-Least significant difference; Values with similar superscripts do not differ significantly at 5% level of significance

The chemical properties of Calophyllum oil tested in five different clones were Acid value, Saponification value, Iodine value and Peroxide value. All the four properties varied significantly across the clones. The Acid value of C-33(17.30) was found to be higher when compared with other clones. C-15 and C-104 were on par with respect to acid value recording 16.70 mg KOH/g. The least acid value was found in the C-26 and C-88(16.53 mg KOH/g) respectively. The presence of low acid value indicates the suitability of the oil as a good lubricant. Phila Raharivelomanana et al. [36] reported that the acidic value of *Calophyllum inophyllum* oil as 13-46mg KOH/g. Sahoo and Das [39] determined the acid value of *C.inophyllum* oil as 44mgKOH/g. The saponification value of Calophyllum oil was highest in clone C-33(197.67) while C-88 recorded the lowest saponification value of 195.67 respectively. The high saponification values of seed oil, indicates the presence of high content of triacylglycerol and impies its utilization in soap making. Vandana et al. [45] reported the saponification value of *Calophyllum inophyllum* oil as 203. In the present study, the highest iodine value was found in the clone C-104 (85.57) followed by C-15 and C-26 (85.53), C-33 with 84.57 and the least in the clone C-88(81.93).The lower the iodine value the better the stability of the oil. Amalia Kartika et al.(2018) reported the iodine value of Calophyllum oil as 82. Phila Raharivelomanana et al. [36] reported that the iodine value of *Calophyllum inophyllum* oil to be in the range of 82 to 98. Among the clones evaluated in this study, clone C-88 ranked the highest for peroxide value (5.267) and the least was seen in clone C-33(4.57). If the peroxide value of an oil is high, it indicates high level of rancidity. The acid value, the saponification value, the peroxide value and iodine value of Calophyllum oil was 42.61 (mgKOH/g oil); 190.650 (mgKOH/g oil); 0.338 (mEq/kg oil) 93 (gI2/100g oil) respectively. Comparing the physico-chemical properties the Clone 15 has been found superior than other clones for biofuel purpose. Clone 15 has not only recorded the highest oil yield, but has also expressed desirable physical and chemical properties of its oil that enhance its biofuel efficiency.

4. CONCLUSION

Clonal variations in oil content and physico-chemical properties of Calophyllum oil showed promising scope for selecting clones for specific end uses such as biofuel purpose, soap making and medicinal purposes. However indepth studies in the respective spheres of use is essential. In this study Clone 15 was found to be an apt clone for biofuel purpose. Clone C-33, with very high saponification value can be utilized for soap making. Oil content being the key end product, more relevance need to be given to the oil yield compared to their physico-chemical properties, in the context of substantial remuneration to farmers. Efforts to minimize unfavourable oil properties such as high acid and peroxide value is essential to increase the shelf life of Calophyllum oil to utilize it for both medicinal and biofuel purpose. It could be predicted that utilizing the wide variations existing in the clones of *C. inophyllum,* in the near future, the underutilized bioresource, *C. inophyllum* can be successfully deployed as a multipurpose oilseed crop and raised as plantations.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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