

Characterization and Physico-Chemical Property of River Red Gum (*Eucalyptus camaldulensis*) Leave Oil

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Abstract

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries. The research is aimed to investigate the physicochemical properties and characterization of *Eucalyptus camaldulensis* leaves oil. The physicochemical were evaluated using the standard procedure and the oil was found to be light yellow color having camphor like smell. The pH of the oil at 22°C was found to be 4.62 while the % oil yield, Optical activity, Refractive index at 27°C, Specific gravity at 27°C, were found to be 49.5±1.021, +34.2°, 1.4384 and 0.812 respectively. The absolute and kinematic viscosity of the leaves oil were 102.30 and 88.20 respectively and the Total acid number (TAN) in mg KOH/g, Iodine number and saponification number were 1.67, 109 and 110.00 respectively. GC-MS revealed the presence of 16 compounds representing 86.96 % of the total oil. The major component was 1,8-cineole (49.08 %), Eucalyptol (34.42) followed by α -pinene (23.90 %), while L-pinocarveol (8.98 %) and globulol (2.71) were the least components. Also α -terpineol, myrtenol, camphene and ciscarveol were present in the oil. The result revealed that *Eucalyptus camaldulensis* leave oil consist mostly of oxygenated monoterpenes that could be very useful for human, animals and industries, also the oil met the FOA/WHO standard of oils. Therefore, these essential oils could be considered as promising substances for development of new drugs. Hence it potential for medicinal and pharmacological uses.

Keywords: Oil, leave, Physico-chemical, chemical and *Eucalyptus camaldulensis*.

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INTRODUCTION

Natural products provide a starting point for new synthetic compounds with diverse structures, and often with multiple stereo-centres that can be challenging [1]. The structural characteristics exhibited by natural products such as chiral centres, aromatic rings, complex ring systems and degree of saturation exhibited by the molecules etc have been shown to be highly relevant to drug discovery efforts [2]. Furthermore, many synthetic and medicinal chemists are exploring the creation of natural product and natural-product-like libraries that combine the structural features of natural products with the compound-generating potential of combinatorial chemistry [3]. Drugs derived from medicinal plants can serve not only as new drugs themselves but also as drug leads suitable for optimization by medicinal and synthetic chemists [4]. The fruit measures approximately 3 by 5 mm. The seeds are gathered directly from the tree and dried in the sun. Viable seeds average 348,000 per kg. The

seeds can be stored up to 9 years if they are placed in hermetic containers at a temperature of 3 to 5°C and a moisture content of 5.5 to 10 percent. *Eucalyptus camaldulensis* medicinal uses include the use of its oil as a remedy for cough and cold. The gum when boiled with water and sugar, become a liquid drink used to treat pulmonary complaints and as a general anesthetic for tooth ache. An infusion of the bark is used as a wash for some eyes, ophthalmia and is highly effective in treating diarrhea [5]. The young shoots are chewed for cold, crushed and placed on sore and cuts. Their infusions are used for the relief of aches, pains, severe headaches, and snake bite. The plant is also believed to be efficacious in the management of high blood pressure. It was also reported to have some antibacterial and antifungal properties [6]. These medicinal properties of *Eucalyptus camaldulensis* inspired its wide spread usage as herbal remedy for the many ailments. The growing interest in herbal medicine demands information on the various plant preparations used [6]. This paper is aimed to characterized and

evaluate the physico-chemical properties of *eucalyptus camaldulensis* leave oil.

MATERIALS AND METHODS

COLLECTION, IDENTIFICATION AND PREPARATION PLANT EXTRACT

The *Eucalyptus camaldulensis* leave were collected from Kazaure Local Government, Jigawa state, Nigeria. It was authenticated at Biological garden of Sciences Laboratory Department, College of Science, Hussaini Adamu Federal Polytechnic, Kazaure, Jigawa state, Nigeria. The leave collected was carefully washed with clean water, and then dried at room temperature. It was pulverized to coarse powder which was used for the extraction of oil by soxhlet extraction method.

Soxhlet Method

A clean, dried 500cm³ round bottom flask containing few anti-bumping granules was weighed (W1). 300cm³ of petroleum ether at 40-60°C was poured into the flask fitted with Soxhlet extraction unit for extraction. The extractor thimble weighing 20 g was fixed into the Soxhlet unit. The round bottom flask and a

condenser were connected to the Soxhlet extractor and cold water was allowed to flow into the condenser. The heating mantle was switched on and the heating rate adjusted until the solvent refluxed at a steady rate. Extraction was carried out for 6 hours. The solvent was recovered and the oil dried in an oven set at 70°C for 1hour. The round bottom flask and oil were weight for further analysis.

Physico-chemical Analysis

Color Determination

Color of the *Eucalyptus camaldulensis* leave oil was determined by physical observation in day light and under ultraviolet radiation of 254 and 366 nm using ultra violet chamber [7].

Odor Determination: Odor of the *Eucalyptus camaldulensis* leave oil was determined by organoleptic evaluation [7].

Determination of Percentage Oil Yield: The percentage yield of *Eucalyptus camaldulensis* leave oil was calculated according to [8].

$$\text{Percentage oil yield (W/W)} = \frac{\text{Weight of oil}}{\text{Total weight of materials used for oil extraction}} \times 100$$

Determination of Optical Rotation

10 ml Polari meter tube containing oil were placed in the tray of the instrument between polarizer and analyzer. Care were taken in filling the tube to avoid the air bubble formation which could disturb the rotation of light. Analyzer were slowly turned until both the halves of the field were viewed through the telescope. The direction of rotation was determined, if the analyzer were turned counter clock wise from the zero position to obtain the final reading, the rotation is levo (-) if clock wise and dextro (+) if anti clockwise.

Determination of Refractive Index

The refractive index of the *Eucalyptus camaldulensis* leave oil sample was determined with the help of Abbe refractometer model A 80251 (BS). Two drops of respective oil were placed on the prism with the help of syringe and the prism will firmly close by tightening the screw head. The apparatus was allowed to stand for 5 min, after that reading were recorded from the display screen [9].

Determination of Specific Gravity

For the determination of specific gravity of *Eucalyptus camaldulensis* leave oil, a clean 50 ml specific gravity bottle were weighted (W0). Then the bottle was filled to the brim with water and stopper was inserted. Extra water spilled out. The water on the stopper and bottle were carefully wiped off and reweighed (W1). Same process was repeated, but using

oil sample instead of water and weighted again (W2). The specific gravity of the oil sample was calculated using the following formula [8].

$$\text{Specific gravity of test sample} = \frac{W_2 - W_0}{W_1 - W_0}$$

Where,

W0 = Weight of empty specific gravity bottle
W1 = Weight of water + specific gravity bottle
W2 = Weight of test sample + specific gravity bottle.

Carbon Residue

A sample of known amount was taken in a silica crucible, heated strongly till the vapours and smoke disappeared, in a sheath iron hood. The sample was then cooled down in a desiccators and cooled down. Carbon residue were then calculated by the following formula according to AOAC [8].

$$\text{Carbon residue (\%)} = \frac{w_1}{w_2} \times 100$$

Where as,

W1= Carbon residue in crucible
W2= Weight of sample

Determination of Viscosity

Viscosity is the resistance to the flow and it was determined by using viscometer. Viscosity plays an important role in determining the structure of liquids.

The viscosity of the *Eucalyptus camaldulensis* leave oil was determined by using viscometer with a selection of spindle number four which was properly fixed to the holder. The container having the oil was carefully placed below the rotor holding the spindle. The spindle was allowed to immerse into the oil inside the container. The meter was turned on and adjusted to a speed of 6 m/s. then the spindle was allowed to rotate in the oil for a period of 30 min until stable reading displayed on the meter's display screen. The viscosity value of the oil was measured in centipoises [8].

Kinematic Viscosity

Kinematic viscosity is the ratio of viscosity to density without any force involvement. It can be obtained by dividing the viscosity of a fluid with its density [10].

$$v = \mu / \rho$$

Where,

v = kinematic viscosity

μ = absolute or dynamic viscosity

ρ = density

Total Acid Number (TAN)

2.5g of *Eucalyptus camaldulensis* leave oil was taken in a flask. 50 ml of methylated spirit was added to the flask, shake well and titrated against 0.1N KOH solution using phenolphthalein as indicator. Alkali was added till a pink color established for a few seconds. The TAN was then calculated using the following formula [8].

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

Where as,

V = volume of potassium hydroxide used

N = normality of Potassium hydroxide

W = weight in g of the sample

Iodine Value Determination

0.2 g of respective *Eucalyptus camaldulensis* leave oil was weighed into a conical flask. 10 ml of carbon tetrachloride and 20 ml of the Wij's solution were added to the flask and the solution was kept in dark for 30 min at room temperature. 15 ml of 10 per cent potassium iodide solution with 100 ml of distilled water were added to the flask. The resulting solution was titrated against 0.1 M sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$), using starch as indicator till the end point where the blue black coloration becomes colorless. A blank titration was carried out at the same time starting with 10 ml carbon tetrachloride. Iodine value was then calculated by the following formula [11].

$$\text{Iodine number} = \frac{\{(B - S) \times N \times 12.69\}}{\text{Weight of the sample}}$$

Where,

B = 0.1 N sodium thiosulfate required (ml) by blank

S = 0.1 N sodium thiosulfate required (ml) by sample

N = Normality of sodium thiosulfate solution.

Determination of the Saponification Value

2 g of *Eucalyptus camaldulensis* leave oil sample was weighted into a clean dried conical flask and 25 ml of alcoholic potassium hydroxide (K (OH)4) were added. A reflux condenser was attached to the flask and heated for an hour with periodic shaking. The appearance of clear solution indicated the completion of saponification. Then 1 ml of 1 % phenolphthalein indicator was added and the hot excess alkali was titrated with 0.5 M hydrochloric acid (HCl) until it reached the end point where it turned colourless. A blank titration was carried out at the same time and under the same condition. The Saponification value was calculated as follows:

$$\text{Saponification value} = \frac{b - a \times 8.05}{M}$$

Where,

b = 0.5 N HCl required (ml) by the blank

a = 0.5 N HCl required (ml) by the sample.

Gas Chromatography – Mass Spectroscopy (GC-MS) Analysis of *Eucalyptus Camaldulensis* Leave Oil

Gas chromatography-mass spectrometry (GC-MS) was performed with GCSM (QP2010 plus Shimadzu, Japan). The analysis was done by gas chromatography with flame ionization detection (GC/FID) and mass spectrometric detection (GC/MS). In the first instance, gas chromatograph (model HP-5890 Series II) equipped with a split-splitless injector, an HP-5 capillary column (25 mm x 0.32 mm, film thickness 0.52 μm) and a flame ionization detector was employed. Hydrogen was used as the carrier gas (1 mL/min). The injector was heated at 250 $^{\circ}\text{C}$, the detector maintained at 300 $^{\circ}\text{C}$, while the column temperature was linearly programmed from 80-280 $^{\circ}\text{C}$ (10 $^{\circ}/\text{min}$ and held at 80 $^{\circ}\text{C}$ (1min), 200 $^{\circ}\text{C}$ (4min) and 280 $^{\circ}\text{C}$ (5min). The GC-MS analysis was performed, using an HP G 1800C Series II GCD analytical system, equipped with an HP-5MS column (30m x 0.25mm x 0.25 μm). Helium was used as a carrier gas (0.9mL/min). The transfer line was heated at 260 $^{\circ}\text{C}$. The EI mass spectra (70eV) were acquired in the scan mode in the mix range 40-600. In each case 1 μL of sample solution in methanol (10 $\mu\text{L}/\text{mL}$) was injected in the split mode (1:30). Identification of constituents was done by matching their mass spectra and retention indices with those obtained from authentic samples and/or NSIT/Wiley spectra libraries, using different types of search (PBM/NIST/AMDIS) and available literature data [12]. The percentage compositions were obtained from electronic integration measurements using flame ionization detection.

STATISTICAL ANALYSIS

The data was statistically analyzed at P-value ($p < 0.05$) significantly accepted and a comparison between the groups was performed using one-way analysis of variance (ANOVA) by Graphpad instat3 software (2000) version 3.05 by Graphpad Inc. The data are given as the mean \pm standard deviation.

RESULTS AND DISCUSSION

Physicochemical Parameters of the Oil of *Eucalyptus Camaldulensis* Leave

The *Eucalyptus camaldulensis* volatile leave oil was obtained by Soxhlet extraction method. The physicochemical properties of the *Eucalyptus camaldulensis* leaves oil such color, odor, density, specific gravity, refractive index, optical rotation, acid value, iodine value, saponification value, viscosity, PH and percentage yield of the oil were evaluated using the standard procedure and the oil was found to be light yellow color having camphor like smell. The pH of the oil at 22°C was found to be 4.62 while the % oil yield, Optical activity, Refractive index at 27°C, Specific gravity at 27°C, were found to be 49.5%, +34.2°, 1.4384 and 0.812 respectively. The absolute and kinematic viscosity of the leaves oil were 102.30 and 88.20 respectively. Finally, the Total acid number (TAN) mg KOH/g, Iodine number and saponification number were 1.67, 109 and 110.00 respectively (Table 1). The percent yield of oils obtained was less than the percentage oil yield of *M. peregrine* seed oil (49.80%) reported from Saudi Arabia [13] and that of egusi, 53.20% but higher in pawpaw and sweet orange seed oils were 40.10% and 43.10% (wt/wt) respectively as reported from northern Nigeria [14]. This may be due to difference in environmental factors and may be due to different in extraction solvent used. This high percentage oil yield of *Eucalyptus Camaldulensis* make it good source of oil for industrial soap production. Also the high level of refractive index by *eucalyptus camaldulensis* leave oil indicated that the oil has high amount of long fatty acid chain.

Specific gravity is the ratio of the density of a respective substance to the density of water at 4°C [15]. Specific gravity values of oils were less than 1 for most of the oils except the oxygenated aromatic compounds [16]. In this study, *Eucalyptus Camaldulensis* leaves oil have high specific gravity values as compared to essential oils from the leaves of *Skimmia laureola* and *Zanthoxylum armatumis* leaves [17].

Acid value gives an indication of the quality of fatty acids in oil. These values however accounted for

the presence of free fatty acids in the oils as an indicator of the presence and extent of hydrolysis by lipolytic enzymes and oxidation [18]. Low acid value in oil indicates that the oil will be stable over a long period of time and protect against rancidity and peroxidation. This could be attributed to presence of natural antioxidants in the leave such as vitamins C and A as well as other possible phytochemical like flavonoids. Acid value is used as an indicator for edibility of an oil and suitability for use in the paint and soap industries [19]. High acid value in oil (e.g. luffa gourd) showed that the oil may not be suitable for use in cooking (edibility), but however, can be used for production of paints, liquid soap and shampoos [19]. The Total acid number (TAN) values recorded in the present study was 1.67 mg KOH/g of oil. These value was found in the permissible limits i.e. 10 mg KOH/g of oil and found to be suitable for dietary purposes, as they contain lower fatty acid contents [20]. This Value obtained was found lower than seed oil of *Jatropha curcas* [21] and seeds oil of five Nigerian species [22]. Hence *Eucalyptus Camaldulensis* leaves oil is a stable and can protect rancidity and peroxidation due to presence of some phytochemicals such as flavonoids etc [18].

The iodine value is a measure of the degree of unsaturation and it is an identity characteristic of leave oils, making it an excellent raw material for soaps cosmetics industries [23]. The iodine value (109 g/100g) obtained in this study was found to be higher when compared with iodine values of *Citrus sinensis* (54.19g/100g). This high iodine value of *Eucalyptus Camaldulensis* leaves oil is an indicator that the oil has higher percentage of unsaturated fatty acids in the leave oil; as such amount of iodine that will be absorbed by the unsaturated acids would be higher [23] and oils with such characteristic may therefore be find useful as raw materials in the manufacture of vegetable oil-based ice cream [24].

The saponification value for the *Eucalyptus Camaldulensis* leaves oil 1 was found to be 110.00 mgKOH/g. The saponification values are higher than the saponification values for egusi, pawpaw and sweet orange seed oils 178.01mgKOH/g, 24.13mgKOH/g and 106.30mgKOH/g respectively [14] and are lower than the saponification value of 213mgKOH/g in neem seed oil and 253mgKOH/g in coconut oil [25]. This is an indication that *Eucalyptus Camaldulensis* leaves oil could be used in soap making. Hence, higher saponification value justifies the usage of fat or oil for soap production.

Table-1: Physicochemical properties of *Eucalyptus camaldulensis* leaves oil obtained by Soxhlet Extraction

S. No	Physico-chemical Characteristics	Results
1	Colour	Colorless to light yellow
2	Odour	Pleasant
3	pH (22 °C)	4.62
4	% oil yield	49.5±1.021
5	Optical activity	+34.2°
6	Refractive index at 27°C	1.4384±0.0001
7	Specific gravity at 27°C	0.812±0.001
8	Carbon residue (%)	2.551±0.041
9	Absolute viscosity	102.30±0.01
10	Kinematic viscosity	88.20±0.06
11	Total acid number (TAN) mg KOH/g	1.67±0.01
12	Iodine number	109±0.13
13	Saponification value (mg KOH/g oil)	110.00±0.57

Result are presented as mean ± SD

Chemical composition of essential oil from *Eucalyptus camaldulensis* Leave Oil

The chemical composition of *Eucalyptus Camaldulensis* leave oil was carried out by Gas Chromatography-Mass Spectroscopy. The GC-MS revealed the presence of 16 compounds representing 86.96 % of the total oil. The major component was 1,8-

cineole (49.08%), Eucalyptol (34.42) followed by α -pinene (23.90%), while L-pinocarveol (8.98%) and globulol (2.71) were the least components. Also α -terpineol, myrtenol, camphene and *ciscarveol* were present in the oil. The *Eucalyptus camadensis* leave oil consist mostly of oxygenated monoterpenes (Table-2).

Table-2: Chemical composition of essential oil from *Eucalyptus camaldulensis* Leave Oil

S/No	Retention Time (mn)	Compounds	%
1.	13.00	Eucalyptol	34.42
2.	13.117	α -Pinene	23.90
3.	13.517	Camphene	0.12
4.	14.460	β -Pinene	0.27
5.	16.323	1,8-cineol	49.08
6.	17.083	α -Campholenal	0.39
7.	18.729	Fenchol	0.16
8.	19.587	L-pinocarveol	8.98
9.	20.233	Borneol	0.36
10.	20.541	4-Terpineol	0.18
11.	20.781	Caren-4-ol	0.15
12.	20.907	α -Terpineol	0.46
13.	21.044	Myrtenol	0.21
14.	21.850	Cis-Carveol	0.17
15.	30.509	Globulol	2.71

Similar study shows that the main components of the leaf essential oil of *E. camaldulensis* var. *petford* were 1,8-cineole, α -pinene, β -pinene, α -terpineol, globulol, borneol, aromadendrene, eudesmol and terpinen-4-ol, Leaf essential oils of *E. camaldulensis* Dehn contained *p*-cymene (68.43%), 1,8-cineole (13.92%), α -pinene (3.45%) and limonene (2.84%), 1,8-cineole (43.00%), α -pinene (5.50%), β -pinene (3.40%), *p*-cymene (5.2%), terpinen-4-ol (3.1%), and globulol (4.1%) as the main constituents while another report shows that ethanone (25.36%), 1,8-cineole (13.73%), β -caryophyllene (11.55%) and carvacrol (9.05%) as the main component of leaf essential oils of *E. camaldulensis* [26]. However, using different extraction procedures. The essential oil of *E. camaldulensis* obtained by supercritical carbon dioxide

extraction had higher amounts of *allo*-aromadendrene and globulol, but has lower quantity of 1,8-cineole, α -pinene, β -pinene, and terpinen-4-ol [27] when compared with this study. Different from this study the leave oil of eucalyptus showed that spathulenol, *p*-cymene and cryptone as main compounds and small quantities of 1,8-cineole. Therefore, these seed essential oils of *E. camaldulensis* from Nigeria could be classified in the chemotype with high 1,8-cineole. The composition of the leaves essential oils of *E. camaldulensis* var. *petford* from Nigeria indicated the predominance of monoterpenes, with 1,8-cineole occurring as the single most abundant constituent.

CONCLUSION

This study revealed that the essential oils from leave *E. camaldulensis* showed quantitative differences of chemicals composition specifically mono-terpenes that could be very useful for human, animals and industries, also the oil met FOA/WHO standard of oils Codex Therefore, these essential oils could be considered as promising substances for development of new drugs. Hence it potential for medicinal and pharmacological uses.

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