Quality Assessment of Eucalyptus Oils Available in Nairobi County, Kenya

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This study assessed eucalyptus oil quality in Nairobi County, Kenya, a product that garnered global attention during the COVID-19 pandemic. Twelve Eucalyptus oil product samples were obtained from local retailers encompassing community pharmacies, supermarkets and health shops. Gas chromatography was utilized for the identification and assay of the primary component, Eucalyptol. Additionally, an evaluation of product attributes, including optical rotation, refractive index, and relative density, was performed on the samples. The examination also extended to product labels and packaging to ensure conformity with established standards. Seven samples adhered to the stipulated labelling and packaging criteria, while two failed the refractive index test, and nine did not meet the relative density specifications. All samples met the optical rotation acceptance criteria. Only one sample conformed to the prescribed content specifications. These findings shed light on the quality of eucalyptus oils and have public health and regulatory implications, particularly given their increased use during the COVID-19 pandemic.

Keywords: Eucalyptus oil, quality, gas chromatography, Eucalyptol

INTRODUCTION

Essential oils are plant extracts usually derived through either hydro distillation or steam distillation from various parts of the plant including leaves, flowers, roots and fruits. Eucalyptus oil is defined by the USP as oil obtained by steam distillation and rectification from the fresh leaves or terminal branchlets of various Eucalyptus species and must contain not less than 70.0% and not more than 95.0% of eucalyptol [1] . However, varying concentrations of *Eucalyptus globulus* essential oil and its constituents have been reported in literature. Although, *Eucalyptus globulus* leaves contain not less than 2% (v/w) essential oil, consisting of not less than 70% (w/w) eucalyptol [2], other studies report some fresh leaves of *Eucalyptus globulus* containing 54% to 61% eucalyptol, 19.5% to 24.3% α-pinene, 6.7% to 9.1% limonene, 2.1% to 5.4% α-terpinyl acetate, and 3.6% to 7.7% sesquiterpenes [3]. This may be attributed the differences observed among the geographical collection regions, different preparation methods to potential hydrolyses during steam distillation. Low content of eucalyptol (35.7%) from fresh *Eucalyptus globulus* leaves containing only 1.87% volatile oil has been reported too [3].

Eucalyptol (1,8-cineole; Figure 1), 1,3,3-trimethyl-2-oxabicyclo [2.2.2]-octane is a biologically active cyclic monoterpenoid ether with the cyclic ether linkage spanning a structure based on cyclohexane. Eucalyptol is also known by a variety of synonyms: cajeputol, 1,8-epoxy-*p*-menthane and 1,8-oxido-*p*-menthane [4].

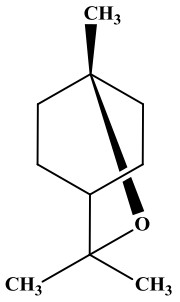

Figure 1. Chemical structure of Eucalyptol.

Eucalyptol has been documented to exhibit various pharmacological effects, including anti-inflammatory, analgesic, antiviral, and antibacterial properties, as reported by several studies [5-10]. It has also demonstrated additional biological effects, such as antimicrobial and antifungal activities [11, 12], insecticidal [13], potential anticarcinogenic attributes [14], antioxidant capacity [15], antinociceptive [16], antispasmodic capacity [17] and antihypertensive activities [18].

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Furthermore, it has found application as a percutaneous penetration enhancer [19]. Clinical utilization of eucalyptol has been documented for conditions like chronic obstructive pulmonary disease [20, 21]. Eucalyptol's industrial applications encompass its use in perfume manufacturing, as an insect repellent, and in soap production [22]. Essential oils, with their historical use as natural remedies, offer distinct advantages, including fewer side effects compared to conventional allopathic medicines [9].

A number of constituents of the *Eucalyptus globulus* essential oil that are of health concern include sensitizers to allergic reactions such as geraniol, hydroperoxides of limonene and linalool. Potential carcinogenic and genotoxic compounds such as myrcene, pinene and quercetin have been reported in the essential oil [23, 24]. Additionally, it has been estimated that about 80% of the essential oils in the market have been adulterated by the addition of synthetic fragments, chemicals and oils [25].

Given the multifaceted antiviral, anti-inflammatory, immunoregulatory, and mucolytic attributes of Eucalyptus oil, its extensive utilization in the context of the COVID-19 pandemic is well-documented. This heightened demand, however, brings to the forefront concerns of potential counterfeiting and adulteration risks [6, 26, 27]. Consequently, it is of paramount importance for nations to rigorously monitor the quality of Eucalyptus essential oil to ensure the safety of consumers. It is noteworthy that exposure to this essential oil can give rise to adverse effects, including gastric discomfort, dizziness, muscle weakness, allergic reactions, and the potential for carcinogenic or genotoxic harm. As such, the primary objective of this study was to undertake a comprehensive assessment of the quality of Eucalyptus oil samples available within the Kenyan market, contributing to the broader discourse on product integrity and consumer safety.

EXPERIMENTAL

Sample Collection

In May 2022, a total of 12 distinct brands of eucalyptus oils were procured from retail outlets, including local community pharmacies, supermarkets, and health and beauty shops, all situated within Nairobi County, Kenya. This sample pool consisted of three specimens obtained from supermarkets, six from community pharmacies, and three from health shops. For each purchase, the smallest available volume of the product was acquired. The samples were coded for blind testing and brand confidentiality purposes.

Reagents

Eucalyptol used as a working reference standard was a kind donation from Glaxo SmithKline (Nairobi, Kenya). The purity of the reference compound exceeded 99%. High-performance grade Dichloromethane used as a diluent was from Merck (Darmstadt, Germany). All other chemicals and reagents utilized in this study were of analytical grade.

Analytical procedures

Visual inspection:

The primary packaging was examined to confirm if the eucalyptus oil was packed in a photoprotective bottle and labelled appropriately. Requirements for completeness of labels included: presence of a manufacture date, expiry date, batch number, instruction for use, warning(s) and the botanical source of the oil.

Physicochemical characterization

Optical rotation of Eucalyptus oil was determined using a Bellingham & Stanley ADP 220 polarimeter (Kent, UK) while the refractive index was determined by using an

Abbe Refractometer (Greifensee, Switzerland). Relative density was determined pycnometrically.

GC-MS analysis

Standard and Sample preparation

A volume of 1 mL was drawn from each of essential oil sample and was mixed with 9 mL dichloromethane and briefly vortexed. Then 1 µL from the resultant mixture was injected into the chromatograph. Eucalyptol working reference standard was similarly treated before chromatography. Triplicate solutions were chromatographed for both samples and the standard.

GC Procedure and Experimental Conditions

Chromatographic conditions: The chromatographic method was optimized using a Shimadzu Corporation Shimadzu QP 2010 gas chromatograph, featuring a split/splitless injector inlet and a flame ionization detector. A Phenomenex ZB-5MS capillary column (5% phenyl-methyl-siloxane, 30 m x 0.32 mm x 0.25 µm) (California, US) was chosen for separation. Nitrogen gas was employed as the carrier gas at a flow rate of 2.2 mL/min. A volume of one µL from each sample was introduced into the chromatograph in split mode (20:1). The injector and detector were operated at temperatures of 250°C and 270°C, respectively. The temperature program extended over 20 minutes, commencing at 75°C (held for 6 minutes), then increasing to 90°C at a rate of 15 ºC/min with a 1-minute hold, followed by an increase to 200°C at a rate of 10 °C/min with a 1-minute hold. To identify eucalyptol, a Shimadzu QP2010 GC-MS gas chromatograph equipped with a mass spectrometer detector from Shimadzu (Tokyo, Japan) was utilized. The same conditions as those in the GC-FID analyses were applied, using a ZB-5 MS capillary column. The ionization voltage was set to 70 eV [28].

Identification of the Eucalyptol: The primary compound, Eucalyptol, was identified by comparing the GC retention time of the samples to that of an authentic eucalyptol standard. Furthermore, the sample spectra were compared to mass spectral data in the NIST 14 and 14s (National Institute of Standards and Technologies, Mass Spectra Libraries), with a focus on similarity index exceeding 98%. All samples were found to contain eucalyptol.

Data analysis

The eucalyptol content of the eucalyptus samples was calculated using one point calibration approach whereby the peak areas of the samples obtained from the analysis were compared to those of the Eucalyptol standard using the following relationship:

$$\text{Content} = A_{sample}/A_{std} \times P_{std} \times C_{std}$$

Where:
A_{sample} is the peak area of the sample;
A_{std} is the peak area of standard;
P_{std} is the Purity of standard; and
C_{std} is the concentration of the standard.

Data analysis was done using Microsoft Excel 2010 where the various peak areas and concentrations were tabulated. The values obtained were compared to USP recommendations, which state that the eucalyptus essential oil must have 1,8-cineole content of not less than 70% and not more than 95%.

RESULTS AND DISCUSSION

Examination of labels and primary packaging

From the 12 samples obtained, 6 (001, 007, 009, 010, 011 and 012) were packaged in an amber-colored glass and one (008) in a cobalt blue glass while the rest of the samples (5) were in clear plastic bottles (Table 1). Those stored in clear bottles did not comply with the British Pharmacopoeia (B.P) 2008 specifications on storage which state that eucalyptus oil should be kept in a well-filled, airtight container, protected from light and at a temperature not exceeding 25^0C. (B.P 2008) [29].

Table 1: Labels and primary packaging of eucalyptus oil samples

Brand Name	Primary Packaging	Date of manufacture	Expiry date	Batch number	Usage instructions	Warning /Precaution	Botanical Name
001	Amber-colored bottle	08/2021	08/2024	Not available	Not available.	Not available.	Not available.
002	Colorless bottle	04/01/22	06/07/24	Available	Use a suitable amount on your body. Massage gently in upward and inward motion. After massaging, wipe off the excess oil with a hot towel.	Store in a cool dry place	Not available.
003	Colorless bottle	12/21	12/22	Not available	Not available.	Not available.	Not available.
004	Colorless bottle	11/20	11/24	Not available	Not available.	Not available.	Not available.
005	Colorless bottle	Not available	31/12/24	Not available	Not available.	Not available.	Not available.
006	Colorless bottle	Not available	Not available	Not available	Not available.	Not available.	Not available.
007	Amber-colored bottle	11/08/2021	11/08/2024	Available	Massage: Add 5 drops of essential oil to 10mL of blending oil. Bath: Add 4 to 6 drops of essential oil to 20mL of blending oil & swirl into water. Vaporizing: Add 6 to 8 drops of essential oil to a vaporizer or burner.	For external use only. Do not use undiluted on the skin. Avoid contact with sensitive areas such as the eyes. Keep out of reach of children. Do not use essential oils on children under 5 years old. Consult your healthcare professional before using essential oils during pregnancy. Best kept in a cool dry place.	*Eucalyptus globulus*
008	Cobalt- blue colored bottle	12/2021	12/2025	Available	Purifying air freshener. Helps breathe easily.	Store in a cool dry place.	*Eucalyptus globulus*
009	Amber-colored bottle	Not available	Not available	Not available	Aromatic: Diffuse using 3-4 drops per 100mL of water or use in personal inhaler. Tropical: Dilute to a maximum of 2-5% in a carrier oil.	Do not use undiluted on skin. Possible skin sensitivity. Keep out of reach of children. Consult a doctor if pregnant or under a physician's care.	*Eucalyptus globulus*

						For external use only.	
010	Amber-colored bottle	14/12/2021	14/12/2022	Not available	Bath: 5-10 drops. Vaporizer: 3-5 drops. Body & Massage: 5 drops – 10mL base oil.	Store in cool & dry conditions.	*Eucalyptus globulus*
011	Amber-colored bottle	14/04/2021	30/03/2023	Available	Baths: Blend 5-8 drops in a teaspoon of carrier oil. Massage: Add 25 drops to 50mL of carrier oil. Vaporization: Add 2-4 drops with water to your oil burner.	Not available	*Eucalyptus globulus*
012	Amber-colored bottle	Not available	Not available	Available	For aromatherapy use. For all other uses, carefully dilute with a carrier oil such as jojoba, grapeseed, olive or almond oil prior to use. Please consult an essential oil book or other professional reference sources for suggested dilution ratios. Add 3 drops of eucalyptus oil, 2 drops each of peppermint oil and tangerine oil to a diffuser and enjoy.	Keep out of reach of children. Do not apply on children's skin, especially the face, mouth, nose and eyes. Avoid contact with eyes. If pregnant or nursing, consult your healthcare practitioner before using. Not for internal use.	*Eucalyptus globulus*

All the samples had an expiry date except samples 006, 009 and 012. Some products had a declared shelf life of 1 year while others were as long as four years. The variation in the shelf life may imply that the declared duration may not be based on any stability studies. Five samples (002, 007, 008, 011, 012) had a batch number while 7 didn't have a batch number, meaning that in case of a product recall, it would be impossible to identify batches. Seven samples (002, 007, 008, 009, 010, 011, 012) had instructions for use. Six samples (002, 007, 008, 009, 010, 012) had a precaution given. The details of the precautions varied greatly. Of the samples of eucalyptus oils purchased, only seven samples (001, 002, 007, 008,011, 012) had KEBS Standardization Mark (SM) stickers.

Optical Rotation, Refractive Index and Relative Density

Determination of the value of optical rotation was based on measuring the angle of the field in which the polarized light was rotated by the oil layer thickness and temperature. Optical rotation values obtained for eucalyptus oil in the study ranged from 0.76 up to -14.68. (Table 2). The B.P specification for optical rotation is 0^0 to $+10^0$ (B.P 2008) [11]. All the samples complied apart from sample 009 which, due to the limited volume, made it difficult for plane polarization to take place [30].

The refractive index was determined based on direct measurement of the angle of refraction oil by maintaining constant temperature conditions. All samples complied with B.P specifications (1.458 to 1.470) apart from two (002 and 005) which had lower values. The B.P specification for relative density is 0.906 to 0.927 (B.P 2008). Only three samples (003, 004 and 006) were within the range for the value of relative density of eucalyptus oil. Two samples (005 and 008) had higher values while 7 samples (001, 002, 007, 009, 010,011 and 012) had lower values. Sample 011 had an extremely low value (0.393)

GC-MS of eucalyptus oil samples

The British Pharmacopoeia specifies that that eucalyptus oil should have a minimum 1,8-cineole content of 70.0%. (B.P 2008) while the Unites States Pharmacopeia specifies that the 1,8-cineole content should not be less than 70.0% and not more than 95.0%. From the results obtained (Table 3), only one sample (Sample 005) passed the quantitative analysis with a 1,8-cineole content of 78.1%. The rest had their content of 1,8-cineole less than the limit set by the pharmacopoeia and hence this shows that these products being sold to the end consumers did not comply with the specifications. Sample 006 recorded a very high content (195.8%). High assay values in medicines, surpassing specified limits, can have adverse implications for both patient safety and treatment efficacy. Elevated levels of active pharmaceutical ingredients may heighten the risk of adverse effects, potentially leading to toxicity. Patients might inadvertently receive excessive dosages, posing risks to their well-being. Moreover, wastage and increased healthcare costs can result from the excess concentration of the active ingredient. Regulatory and legal challenges may also arise as well as brand reputation damage, and a loss of trust in the pharmaceutical industry and healthcare system. In severe cases, market withdrawal may be necessary..

Table 2: Physicochemical attributes of Eucalyptus oil samples

Eucalyptus oil sample	Optical Rotation (^{0})	Refractive Index	Relative Density
Sample 001	0.76	1.4620	0.808
Sample 002	0.09	1.3860	0.853
Sample 003	4.63	1.4600	0.919
Sample 004	4.51	1.4640	0.911
Sample 005	1.14	1.4330	1.041
Sample 006	3.29	1.4600	0.919
Sample 007	6.01	1.4610	0.843
Sample 008	7.17	1.4590	0.967
Sample 009	14.68	1.4650	0.824
Sample 010	3.16	1.4600	0.860
Sample 011	0.67	1.4610	0.393
Sample 012	5.15	1.4610	0.864

Table 3: Eucalyptol content in samples collected.

Sample	Eucalyptol content (% v/v)
Sample 001	32.3
Sample 002	0.3
Sample 003	48.3
Sample 004	53.1
Sample 005	78.1
Sample 006	195.8
Sample 007	68.4
Sample 008	59.7
Sample 009	38.1
Sample 010	47.9
Sample 011	23.7
Sample 012	27.8

Sample 002 had an extremely low content (0.3%). Low assay values in medicaments may lead to reduced treatment efficacy, ineffective therapy, prolonged illness, and risks of disease progression. It can mislead patients and healthcare providers, potentially leading to safety concerns, legal issues, and a loss of trust in the pharmaceutical industry. Additionally, it can have economic implications due to wasted healthcare resources.

CONCLUSION

In summary, the analysis of 12 samples revealed that only one (8%) met the specifications outlined in BP 2008 for eucalyptol content. None of the samples satisfied all the pharmacopeial requirements for content, optical rotation, specific gravity, and refractive index. Notably, Sample 002, failed multiple quality tests namely content, refractive index, and relative density, despite bearing the Kenya Bureau of Standards standardization mark. These findings underscore a prevailing issue of substandard product quality, even within the limited sample pool employed in this study, which raises concerns about the effectiveness of regulatory systems for borderline medical products. The presence of poor-quality products inherently poses significant risks to public health. Consequently, there is need for rigorous quality control, the establishment, enforcement, and adherence to regulatory standards concerning Eucalyptus oil products and other health-related supplements to ensure the safety and well-being of the public.

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