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Optimization of Essential Oil Extraction from *Eucalyptus grandis* Leaves by Clevenger Distillation

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Abstract: ***Recent statistics have shown a rise in the margin of consumption and utilization of essential oils, over the past years this has been marked by a divergence in the dependence of allophaticallopathic drugs. However, essential oil extraction processes have been observed to suffer from minimum output levels. The purpose of this study was to investigate the effects of changing the process parameters (time of extraction, volume of water vs quantity of plant material) on the quantity of the extracted essential oil. A bulk sample of fresh Eucalyptus grandis leaves was collected from a single location in a reserved garden in Kwale County, of Kenya. Proper identification was done and then the leaves were ground to a fine powder. The essential oil was extracted using the distillation method. Designed experiments were carried out to map out quantitative effects of the different operating parameters (time of extraction and water: plant material ration) on the yield of the ethereal oil from the ground sample. To test the effect of time of extraction and thus temperature, time of extraction was varied from 30 to 210 minutes using 30 minutes intervals then studying the % yield. Water volume was varied from 300 ml to 700 ml using an interval of 100 ml. The qualitative analysis of the extracted oil was done using gas chromatogram-Mass spectrometer to identify the specific components. According to the results, essential oil yields at the shorter distillation times (DT) was low and generally increased with increasing DT with the maximum yields achieved at 150 min. Increasing the amount of water directly increased the number of water molecules evaporating and this increased the total pressure of the system. The high boiling constituents were now easily extracted at a lower boiling point resulting to increase in the oil yield. However, this was up to a volume of500ml. GC-MS chromatogram of the Eucalyptus grandis essential oil showed the oil possessed compounds with known biochemical properties applicable in the medicinal world. These compounds have individual antifungal and/or antibacterial properties capabilities but their effectiveness could be enhanced synergistically. The study dwelled on time of extraction and volume of water vs. amount of plant material. Future studies should focus on the variation of pressure and temperature as determinants of the quantity and quality of the ethereal oil from plant materials.***

Keywords: Essential Oils, *Eucalyptus grandis*, Clevenger Distillation, Optimization

1. Introduction

At present, the intersection of cutting edge technology and folklore wisdom has enabled scientists to procure most of their raw materials from more available and natural occurring resources especially forests. This is certainly substantiated by the current upsurge in the demand of essential oils in the international markets (Reichling *et al.*, 2009).

Essentials oils also known as ethereal oils are usually extracted from different parts (leaves, flowers, seeds, bark, roots) of a wide variety of plants (Lawless, 2013). A guess estimated 4000 essential oils are known of which approximately 500 are of commercial importance (Burt, 2004; Lawless, 2013). Essential oils have been conventionally used as key raw materials in perfumes and flavorings primarily because of their fragrance and high volatility nature (Berger, 2007).Nevertheless, these oils have currently found utility in the medicinal arena principally as insect repellents or insecticides (Bakkali *et al.*, 2008).

With the increasing demand of essential oils in the world presently, numerous species of trees previously exploited as source of timber have now been studied to find out if they have any ethereal oils (Sartoratto *et al.*, 2004). These efforts have often yielded good results with some parts of many trees having been found to produce significant, amounts of oil which has diverse applications (depending on the species of trees) in many fields.

Of interest are the new volatile oils from *Eucalyptus spp*.The *Eucalyptus* is a diverse genus of flowering in the myrtle family, Myrtaceae. According to the literature, approximately3800 species are found within this family appearing in 140 genera and occurring along tropical and subtropical regions of the world.

Eucalyptus oil has numerous applications from pharmaceutical, antiseptic, propellant, flavoring and fragrance (Lawless, 2013).Commercially important essential oils from *eucalyptus spp* can be grouped according to their uses into three classes; medicinal, industrial and perfumery oils (Maciel *et al.*, 2010). Perfumery oils account for only a small fraction while medicinal and industrial oils are used in about equal proportions. The most common types of ethereal oils from eucalyptus spp come from *Eucalyptus polybractea* (Blue Mallee), *Eucalyptus globulus* (Blue gum), *Eucalyptus radiate (*arrow-leaved peppermint)and *Eucalyptus citriodora* (lemon-scented gum).

The *Eucalyptus* oils are rich in potent therapeutic compounds such as cineol, citronellal and α-phellandrene and thus have many therapeutic applications especially in healing pain and inflammation of respiratory tract mucous membranes, coughs, asthma, bronchitis, sinus pain and inflammation, and respiratory infections (Silva *et al.*, 2003;

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Prabuseenivasan *et al.,* 2006). They are also used as an antiseptic, insect repellent, and treatment option for wounds, burns, and ulcer (Batish *et al.,* 2008; Trongtokit *et al.,* 2005).Eucalyptus oils are also popularly used as a fragrance in perfumes and cosmetics, and are found in mouthwashes, liniments and ointments, toothpastes, cough drops, and lozenges. They are commonly mixed with other oils to make them more easily absorbed by human skin. This supports the moisturizing process, which explains the oil's presence in skin products like a natural sunscreen (Lawless, 2013; Breitmaier, 2006).

The chemical composition of any essential oil is so imperative in ascertain ingits quality and consequently price in the market. This call for insistent improvement of the quality of the essential oils produced to attain a competitive and profitable market (Zhang& Li, 2010). It is thus necessary to find the best extraction approach, which could provide the noblest chemical component and best quality of essential oils. Since distillation is the approved means of extraction of essential oils, it is imperative to note and understand and alter different distillation parameters such as temperature, pressure, time of extraction to ensure the quality and yield of essential oil are maximal.

This research aimed to optimize essential oil extraction from *eucalyptus grandis* leaves by the use of Clevenger distillation. This involved changing the process parameters (time of extraction, water: plant material ratio) and establishing their overall contribution to maximizing the process of extraction and also the composition of *Eucalyptus grandis* oil.

2. Materials and Methods

Collection and preparations of the plant material

A bulk sample of fresh *Eucalyptus grandis* leaves was collected from a single location in a reserved garden in Kwale County of Kenya. Proper identification was done at the Herbarium of the University of Nairobi. The fresh plant material was taken to the laboratory, where the leaves were picked off the stalks and mixed thoroughly to form a homogeneous and representative stockpile sample. The leaves were air dried under a shade for 48hrs. After the leaves were sufficiently dried, they were ground to a fine powder.

Extraction of the Oil

40g of ground eucalyptus leaves were added into a clean round bottom flask and 500ml of distilled water added. The round bottom flask together with its contents was placed in a heating mantle of equivalent volume (i.e. using a 1litre round bottom flask would require either a 1 litre or 2 litre heating mantle). The round bottom flask was then connected to the other distillation apparatus: Clevenger apparatuses, condenser. The water in the round bottom flask was heated to boiling. As soon as the boiling begun, the temperature was regulated to a point where there was controlled boiling.

Isolation of the oil extracts from the steam condensation

Essential oils and water condensate are known to have different densities and form an immiscible two liquid phase mixture at low room temperature conditions. The separation of the essential oils from the condensate hence utilizes this density and immiscibility advantage for the two to be isolated from each other. A calibration mark on the separating funnel acts as the reference point during the emptying and determination of the mass of the decanter contents.

In separating the water from the oil, the water layer was carefully run out from the bottom of the separating funnel by opening the tap until its meniscus was just at the calibration mark. The contents that remained inside the separating funnel were the oil layer and the water between the tap bridge and the bottom of the calibration mark.

The purified ethereal oil sample was placed in a dry beaker and an extraction solvent (hexane) was added. The extract obtained was dried over anhydrous sodium sulphate and placed in a dry vial (pre-weighed). The vial was covered with a punctured aluminum foil (to allow evaporation of hexane) and stored for a night.

The contents of the vial were weighed using an electronic analytical balance. The difference in mass of the vial plus the oil and the mass of the empty vial would be the mass of the oil. The yield of extraction is defined as the ratio of the mass of essential oil collected after evaporation of the solvents and the mass of leaves dry matter. The oil sample was stored in a refrigerator and kept at temperature of 4^{o}C for further analysis.

Quantitative characterization of the eucalyptus essential oils extracts

Designed experiments were carried out to map out quantitative effects of the different operating parameters (temperature, pressure and time of extraction) on the yield of the ethereal oil from the ground sample. To test the effect of time of extraction and thus temperature, time of extraction was varied from 30 to 210 minutes using 30 minutes intervals then studying the % yield. Water volume was varied from 300 ml to 700 ml using an interval of 100 ml.

The yields of the extraction were calculated from the relation between the mass of the essential oil extracted and the raw material mass used in the extraction. In this study 40 grams was used per extraction.

$$Oil\ Yield\ (\%) = \frac{(Mass\ of\ vial\ + Oil) - (Mass\ of\ vial)}{Mass\ of\ leaves\ sample\ used}\ x\ 100$$

The qualitative analysis of the oil

The qualitative analysis of the extracted oil was done using gas chromatogram-Mass spectrometer to identify the specific components.

3. Results and Discussion

Time of extraction

Time of extraction was varied from 30 to 210 minutes and the % yield determined. The results are tabulated below.

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Table 1: Variation of Extraction time

Time/duration of extraction	Amount of water used in (ml)	Average mass of oil extracted per amount of Ground leaves used (g)	% oil yield
30	500	0.2105/40.003	0.5262
60	500	0.231/40.014	0.5773
90	500	0.2391/40.014	0.5975
120	500	0.248/40.007	0.6199
150	500	0.2995/40.002	0.7487
180	500	0.2897/40.007	0.7241
210	500	0.292/40.007	0.7299

From the table 1, it can be clearly stated that extraction at 150minutes was found to be the optimal extraction time.

Water to plant material ratio

Different water amounts between 300ml to 700ml were added to 40g of plant material.700 being the limit (optimum reactor volume). The results are tabulated below:

Table 2: Raw data for Water to plant material ratio

Volume of Water	*Average mass of oil extracted per amount of Ground leaves used (g)*
300 ml	0.242/40.007 (0.6049%)
350 ml	0.252/40.007 (0.63%)
400ml	0.287/40.004 (0.7174%)
500ml	0.302/40.003 (0.7549%)
600ml	0.299/40.007 (0.7473%)
700ml	0.304/40.002 (0.76%)

The results indicate that optimal yield was attained when 700 ml of water was used for the extraction process.

Physical Description of the Oil

The oil obtained was clear golden yellow color with a camphoreous smell (Characteristic of eucalyptus oil). The Refractive index attained for the oil was1.4345 which was quite high indicating good quality.

Qualitative Analysis

Data obtained from GC-MS (figure 1) showed the following compounds:

- $C_{17}H_{28}O_2$- (Nerolydil acetate/1,3,3-trimethyl acetate)
- $C_{15}H_{26}O$- (Globulol)
- $C_{10}H_{18}O$- (2-oxabicyclo (2,2,2) octane, I, 3,3-trimethyl/eucalyptol)
- $C_{11}H_{18}$- (1,4a-methyl-1, 2,3,4,4a, 5,6,7-octahydro-4a-methyl)
- $C_{15}H_{26}O$- (2-naphthalenemethanol/β-Eudesmol)
- $C_{15}H_{24}$-Aromadendrene
- $C_{10}H_{18}O$- (1-Terpineol/3-cyclohexen-1-ol)
- $C_{10}H_{16}O$-2-Pinen-10-ol/myrtenol/Bicyclo (3,1,1) het-2-ene-2-methanol)
- $C_{12}H_{20}O_{2-}$ (α-Terpineol acetate)
- ($C_{12}H_{20}O_2$- (3-cyclohexane-1-methanol-4-trimethyl acetate),
- $C_{10}H_{18}O_2$- (2,10-Bornanediol-oxo-)
- ,$C_{11}H_{18}$ (2,2-Dimethyl-3-vinyl-bicyclo (2,2,1)heptane,
- $C_{10}H_{14}O$-Carvone
- ,$C_{12}H_{20}O_3$- (2-oxabicyclo (2,2,2) octa-6-ol, 1,3,3-trimethyl acetate,
- $C_{15}H_{24}$- (1H-cycloprop (e) azulene),
- $C_{15}H_{26}O$- (Globulol),$C_{15}H_{26}O$- (2-naphthalenemethanol),
- $C_{15}H_{22}O$- (4,6,6-Trimethyl-2- (3-methylbuta-1,3-dienyl)-3-oxatricyclo (5.1.0.0 (2,4))octane,
- $C_{16}H_{24}O_2$- (2-methyl-2- (2,6,6-trimethyl-3-methylene-cyclohex-1-enyl)- (1,3)dioxolane,
- $C_{14}H_{22}O_2$- (3-Buten-2-one),$C_{10}H_{18}O$- (isoborneol),
- $C_{15}H_{24}$- (naphthalene),$C_{16}H_{24}O_2$- (3,5-di-tert-butyl-4-hydroxyacetophenone),
- $C_{15}H_{26}O$- (1H-Benzocyclohepten-7-ol,
- $C_{15}H_{24}$-Azulene,$C_{11}H_{18}$- (cyclohexene),
- $C_{10}H_{18}O$- (Bicyclo (2,2,1) heptan-2-ol)

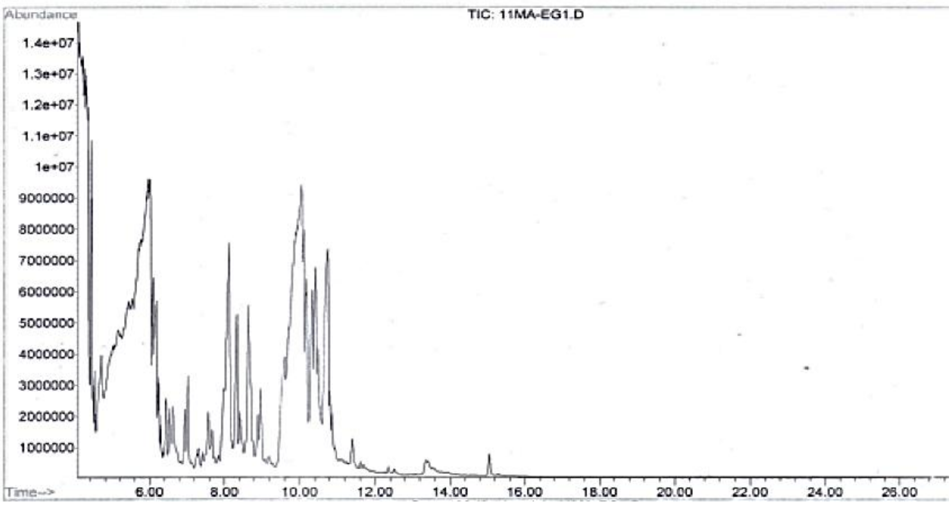

Figure 1: GC chromatogram (Ground leaves extract)

4. Discussion

The effects of operating parameters (temperature, volume of extraction water and time of extraction) on the quantity of essential oil extracts by means of steam extraction were studied using eucalyptus leaves as the plant material.

According to the results, a number of factors individually play a significant role in the quantity and quality of essential oils extracted from plant materials. Time of extraction is known to affect the chemical composition of the essential oil as most compounds in the oil have different boiling points. Low boiling point constituents such as α-Pinene, camphene, β-Pinene, myrcene, Para-cymene, limonene, and γ-terpinene

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are found to be concentrated at low duration time of extraction, while compounds of high boiling points such as geraniol and geranyl-acetate at high duration times. In this study, the results showed that essential oil yields at the shorter distillation times (DT) was low and generally increased with increasing DT with the maximum yields achieved at 150 min. Increasing the extraction time gives more time for the heavier and high boiling point constituents to diffuse out of the oil pockets in the leaves thereby increasing the yield significantly. However, higher duration time (180mins) suffers minimum oil yields. This is due to evaporation of the oil being collected as a result of inadequate cooling time of the apparatus. And so the hot apparatus serve as source of heat causing the oil collected to evaporate. This is evident from the line graph plot.

Condition of extraction plant material is known to affect the yield of the oil. Ground leaves were used for the analysis of finding the optimal extraction time due to their high surface area for extraction compared to the sliced and whole leaves. Amount of water to plant material ratio was adjusted to find out the optimal ratio. Volumes of distilled water were taken from 300 ml being the minimum amount and 700 ml being the maximum:

- 300ml was the minimum since the plant material needed to be partially immersed in the water for direct steam generation, which is the basis of Clevenger distillation.
- 700 ml the maximum so to achieve optimal reactor volume. The yield of oil increased with increase in amount of water with constant mass of leaves up to 500ml where there was no significant increase. The ratio 1:12.5, which represents (40g in 500ml of water) was found to be the optimal ratio.

Increasing the amount of water directly increased the number of water molecules evaporating and this increased the total pressure of the system. The high boiling constituents were now easily extracted at a lower boiling point resulting to increase in the oil yield. However, this was up to a certain volume (500ml). Adding any amount of water resulted to equilibrium between the water and water vapor due to small reactor volume hence no significant increase in oil yield was observed.

The results from the GC-MS have also shown medicinal potential in the oil, as compounds with anti-microbial activity were found in the oil, which also matched literature sources.

5. Conclusion

Time of extraction proved to be a crucial parameter in distillation with 150 minutes being the optimal time frame. The yield of the oil was observed to have a positive correlation with increase in time. Ratio of water to plant material was found to have a considerable effect on the quantity of the oil extracted and no effect on the quality of the oil. A ratio of 40g in 500ml of water was found optimal, as there was no further significant increase in the yield of the essential oil with increase of amount of water. The *Eucalyptus grandis* essential oil was found to possess compounds with known biochemical properties applicable in the medicinal world. These compounds have individual antifungal and/or antibacterial properties capabilities but their effectiveness could be enhanced synergistically.

6. Recommendations

The study dwelled on time of extraction and volume of water vs. amount of plant material. Future studies should focus on the variation of pressure and temperature as determinants of the quantity and quality of the ethereal oil from plant materials.
The effect of time of extraction, volume of solvent, temperature and pressure should also be studied under different extraction methodologies such as microwave extraction, supercritical extraction and so on.

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