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Comparative Study on Extraction and Formulation of Perfume Oil from Lemongrass (Cymbopogon Citratus) by Solvent Extraction and Steam Distilation

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Abstract

With increasing uses, rising world population and improved standard of living, the demand for essential oils increases. Essential oil can be extracted from Lemongrass (*cymbopogon citratus*), using different extraction methods. In this work solvent extraction and steam distillation methods were used to extract essential oil from lemongrass. Solvent extraction method yielded 5.00%, and steam distillation methods yielded 2.21% essential oil respectively. From the experiment solvent extraction gave the highest yield because of the less exposure air and heat. Physical and chemical properties were assessed to evaluate quality characteristics of the oils. Results are shown in this wok, saponification value of 140mgKOH/g and 154.28mgKOH/g (steam distillation and solvent extraction), FFA of 1.4 and 2.4, Acid value of 2.8ml/g and 4.8ml/g, Peroxide value of 3.5meq/kg and 4.5meq/kg. The result from these analysis of these essential oils have shown that they can be applied in vast industrial processes such as soap making due to their high saponification values. Formulation of perfume oil from lemongrass was carried out. From the t-test and regression statistical analysis done t-stat gives 1.8439 and p-value two-tail is 0.1624.

Keywords: Cymbopogon Citratus, Solvent Extraction, Steam Distillation, Essential Oil

Introduction

The word perfume is used today to describe scented mixtures and is derived from the Latin "per" meaning "through" and "fume," or "smoke, it is a fragrant liquid that is sprayed or rubbed on the skin or clothes to give a pleasant smell. Extraction of perfume from various plants resources is of ancient origin Oloyede (2009). In fact the natives from different tropical regions of the globe have long been extracting oil from numerous oil bearing plants. Human beings since the ancient time have known how to extract oil from their natural resources. While fragrant liquids used for the body are often considered perfume, true perfumes are defined as extracts or essences and contain a percentage of oil distilled in alcohol.

Lemongrass belong to the Family Poaceae (Gramineae), Cymbopogon species is used in herbal teas and other nonalcoholic beverages in baked goods, and in confections. Oil from lemongrass is widely used as a fragrance in perfumes and cosmetics, such as soaps and creams. Citral, extracted from the oil, is used in flavoring soft drinks in scenting soaps and detergents, as a fragrance in perfumes and cosmetics, and as a mask for disagreeable odors in several industrial products. Citral is also used in the synthesis of ionone used in perfumes and cosmetics. Biological research has shown that various chemical compounds of lemongrass essential oil exhibit antimicrobial (Bassolé *et al*., 2011), antioxidant (Mansour *et al*., 2015), antiparasitic (Kpoviesi *et al*., 2014), insecticidal, and insect repellent activities (Brugger *et al*., 2019). The genus has about 55 species, most of which are native to South Asia, Southeast Asia and Australia. Ranitha *et al*. (2014) [14]. Two major types have considerable relevance for commercial use: East Indian lemongrass (Cymbopogon flexuous) is native to India, Sri Lanka, Burma and Thailand, whereas West Indian lemongrass (Cymbopogon citratus) is assumed to originate in Malaysia. The reported life zone for lemongrass is 18 to 29 degrees centigrade with an annual precipitation of 0.7 to 4.1 meters with a soil pH of 5.0 to 5.8 (East Indian) or 4.3 to 8.4 (West Indian). Since the plants rarely flower or set seed, propagation is by root or plant division. The plants are harvested mechanically or by hand about four times each year with the productive populations lasting between four and eight years Extensive breeding programs have developed many varieties of lemongrass.

Methodology

Fig 1: Shows Soxhlet Extraction

Fig 1.1: Shows Steam Distillation

Raw Material and Reagent Collection

Fresh Lemongrass sample was collected from the garden in the early hours of the morning. The sample was taken to the laboratory, where the leaves were cut into slices to reveal the tighter inner stem and was weighed to be 300g of lemongrass.

50g of dried Lemongrass leaves was crushed and placed in a Soxhlet extraction apparatus with 300ml of n-hexane. The solution was gently refluxed using a filter paper, the solvent (N-Hexane) in the round bottom flask is heated and cooled in the condenser into the reflux which extract the essential oil, and it get to a height where it pass through a tube back to the round bottom flask. These continue for three hours and allowed to cool to room temperature. The process was repeated five times. The liquid containing the mixture of N-Hexane and essential oil was then distilled further where the temperature was adjusted to 67^{o}C. The remaining oily liquid in the flask was the essential oil, it was weighed and the difference between the final weight of the beaker with extract and the initial weight of the empty beaker gave the weight of essential oil.

Steam from boiling water is passed through the raw material for 180 minutes, which drives out most of their volatile fragrant compounds. The condensate from distillation, which contains both water and the aromatics, is settled in a separating funnel. This allows for the easy separation of the fragrant oils from the water as the oil will float to the top of the distillate where it is removed, leaving behind the watery distillate. This was done for five batches, the water collected from the condensate, which retains some of the fragrant compounds and oils from the raw material, is called hydrosol and is sometimes sold for consumer and commercial use. This method is most commonly used for fresh plant materials such as flowers, leaves, and stems.

Physical characterization is determining the physical properties of the extracted oil. Some of these properties are specific gravity, density etc. Chemical properties of lemongrass oil are such as acidic value, saponification value. Physicochemical properties were used to determine the quality of oil extracted.

Determination of pH

About 1g of the lemongrass oil was poured into a clean dry 25ml beaker; next 10ml of distilled water was added in the beaker and stirred slowly. It was then cooled in a cold-water bath to 25^{0}C. The pH electrode was standardized with buffer solution then the electrode was immersed into the sample finally the pH value was read and recorded.

Determination of Specific Gravity

The specific gravity determination is carried out in order to ascertain the density of the oil sample (Lemongrass). The specific gravity of the essential oil was determined using a pycnometer. The weight of the empty pycnometer was recorded; 2ml of water was poured into the pycnometer and the weight was recorded too. The specific gravity was calculated using the formula;

$$\text{S.G} = \frac{\textit{weight of empty pycnometer} - \textit{weight of pycnometer} + \textit{essential oil}}{\textit{weight of empty pycnometer} - \textit{weight of pycnometer} + \textit{distiled water}} \quad (3.1)$$

Determination of Density:

The density of the essential oil was calculated using the formula below:

$$\text{Density} = \text{specific gravity} \times 1000\text{kg/m}^3 \quad (3.2)$$

Determination of Free Fatty Acid

The free fatty acid of the essential oil was determined by using titrimetric method using ethanol and phenolphthalein. 1g of lemongrass oil was accurately weighted and dissolved in 10ml of 95% ethanol and 1-2 drop of phenolphthalein indicator was added. The free acid was then titrated with standard 0.1 normality of aqueous sodium hydroxide solution by adding the alkali drop-wise at a uniform rate. The content of the flask was continuously agitated. The primary manifestation of the pink coloration that did not fade with 10sec was considered the end point. The formula for determination of free fatty acid value (FFA %) is given as:

$$\text{Free fatty acid (FFA \%)} = \frac{(v-s)xNxM}{W} \quad (3.3)$$

Where,

v = volume blank titer, N = Molarity of acid used, M = Molecular weight of acid, w = Weight of oil sample used s= Sample titer value

Determination of Saponification Value Using Experimental Procedure

25ml of ethanolic potassium hydroxide solution was measured into a round bottom flask and 1g of oil sample (lemongrass) was dissolved in it. A reflux condenser was attached to the flask and heated for 30 minutes. 1-2 drops of phenolphthalein solution was added and titrated against 0.5M hydrochloric acid solution to a point where the pink colour disappeared. A blank titration was conducted under the same conditions (omitting the oil). The formula for determination of saponification value is given as:

$$\text{Saponification value (SV)} = \frac{(B-S)xM}{W} \quad (3.4)$$

Where,

S = Sample titer value, B = Blank titer value, N = Normality of acid used, M = Molecular weight of KOH used, W = Weight of oil sample used

Determination of Peroxide Value Using Experimental Procedure

One (1) gram of the essential oil was weighed into a clean dry boiling tube. 1ml potassium iodide and 30ml of solvent mixture (3 vol glacial acetic acid + 2 vol chloroform), were added, add 30ml distil water, shake and mix well for one minute, add 2-3 drops of 1% starch as indicator and titrated with 0.01N sodium thiosulphate solution.

Formulation of Perfume with Essential Oil Produced

Apparatus and Reagents

1. Pipette
2. Funnel
3. 50ml and 120ml beakers
4. Perfume bottle
5. Methanol
6. Distilled water
7. Lemongrass essential oil

Table 1.0: Basic perfume formulation

Ingredients	%	ml
Methanol	68	10.2
Essential Oil	20	3.0
Water	10	1.5
Sunflower oil	2	0.3
Total	100	15

Procedure

1. 10.2ml of Methanol was measured into a 50ml beaker.
2. 1.5ml of water was measured and added to the methanol in the beakers.
3. This was followed by the addition of 0.3ml of sunflower oil into the mixture.
4. The mixture was thoroughly mixed together for homogeneity.
5. Finally the 3.0ml essential or fragrance oil was added to the mixture and stirred.
6. The resultant perfume of 15 ml was put inside a bottle and covered.

Table 1.1: Shows weight of oil with respect to time for steam distillation method

Weight of oil (g) (Steam Distillation)	Weight of oil (g) (Soxhlet)	Time (mins)	Mass of leave used(g)	Volume of solvent used (ml)
2.30	4.80	180	100	350
4.02	11.00	360	100	350
6.64	15.01	540	100	350

Table 1.2: Physicochemical Characteristics of Lemongrass Essential Oil Compared To Standard

S. No	Characteristics	Steam distillation	Solvent extraction (soxhlet)	Standard
1	Specific gravity	0.8950	0.8750	0.899 Less than 1
2	Density (kg/m^3)	895.0	8750	825.0 – 925.0
3	Free fatty acid	1.4	2.4	0-1 (% oleic acid)
4	Acid value	2.8 ml/g	4.8 ml/g	0.56 0 – 4 (% oleic acid)
5	Peroxide value (meq/kg)	3.5	4.5	3.4 0 – 7.5
6	Saponification value (mgKOH/g)	140.25	154.28	151.7 Less than 188-196

Discussion of the Perfume Oil Produced

About 0.3ml of carrier oil (Sunflower oil) were first placed in a clean and sterilized beaker to avoid unexpected reaction, Followed by Methanol (10.2ml). 1ml of lavender were added as the base note in the beaker, then 1.5 ml of lemongrass oil as the middle note were also added and lastly 0.5ml of orange as top note. The beaker containing mixture was covered and stirred for 15mintues to allow the scent to mingle. Then 1.5ml of distilled water was added to keep the evaporation of perfume a little low. The mixture was thoroughly mixed together for homogeneity, the resultant perfume was then placed in a bottle using a funnel, covered and placed in a cool dark place for weeks in order to marry and mature. For the purpose of this project, a 15 ml perfume was produced according the formulation.

Discussion of Analysis

Physicochemical properties of the essential oil extracted as shown in tables, shows the following:

1. **Effect of extraction time on the yield of extracted oil:** Extraction time plays a great role on the percentage yield of lemongrass oil using both solvent extraction and steam distillation in figures show that as contact time increase the oil yield also increase till transfer of oil from leaves to steam attain zero. When the maximum amount of extractable oil is obtained, the oil yield level remains invariable even by extending the reaction time. The yield from the extraction of essential oil was 5.00% and 2.21%, using solvent extraction method and steam distillation method respectively.
2. **Effect of particle size on the yield of extracted oil:** from the experiment, it was observed that particle size had an effect on the oil yield. Size reduction maximizes the surface area, which in turn enhances the mass transfer of active principle from plant materials to solvent. It was observed that the minimum particle size has had maximum oil yield whereas the maximum

particle size has had minimum oil yield.

From the experiment carried out, I observed that for higher yield of extracted oil solvent extraction method was best, but the demerit is essential oil produced with solvent extraction method is not usually used for perfume production because of the pigments extracted with the essential oil. While steam distillation method, the extracted oil contain no pigments, the yield of the oil extracted was lower than that of the solvent extraction. This conforms to works done by other researchers.

The extracted essential oil from lemongrass at room temperature using steam distillation method was pale yellow, with pungent lemon odour and has a cold nature. Because of its high volatility, it was stored in a well filled air-tight container confined from light in cool place (Basavaraja *et al*., 2011). The essential oil was insoluble in water, miscible in alcohol and in oil. While the extracted essential oil from lemongrass at room temperature using solvent extraction method was dark green, with mild lemon odour due to the presences of pigments.

The specific gravity was 0.895 and 0.875 for steam distillation and solvent extraction method respectively; it is similar to the values obtained by Barkatullah *el al.,* (2012). The specific gravity was less than one (l) and so indicates that the lemon grass essential oil had low thickness property and so flowed easily. Similar values were obtained by Akinyeyo, Adeyeye, Fosakin and Agboola (2011) [2].

The acid value obtained from this experiment was 2.8ml/g and 4.8ml/g for steam distillation and solvent extraction method respectively. Acid index indicated the amount of free acid in the essential oil and depends on the extraction method and the freshness of the raw material. When being preserved for a long time, the acid index of the materials increased due to oxidation and esterification in the degraded essential oil.

The saponification value was 140.25mgKOH/g and 154.28mgKOH/g for steam distillation and solvent extraction method respectively. The saponification value is in the range reported for plants that is less than 188-196 (Barkatullah, Muhammad, Abdur and Inyt-Ur, 2012). The rather low saponification value suggests that the oil combined high proportion of high molecular weight fatty acids and had low level of unsaturation (Olaniyi *et al.,* 2014).

Peroxide value is used as an indicator of deterioration of oil. The peroxide value of the oil was 3.5meq/kg and 4.5meq/kg for steam distillation and solvent extraction method respectively, was quite low indicating low oxidative rancidity and high level of antioxidants in the lemon grass essential oil (Kyari, 2008).

The perfume produced using the lemon grass essential oil had a strong, long-lasting, and pleasant smell. This is agreement with the report of Mane *et al* (2015). When applied to clothing, the perfume did not leave any stain but when applied directly on human skin, it caused itching minutes after application.

Data Analysis

Table 1.3: Show the Percentage Oil Yield

	Steam distillation	Solvent extraction (soxhlet)
Volume (ml)	1050	1050
Mass(g)	300	300
Oil yield (%)	2.21	5.00

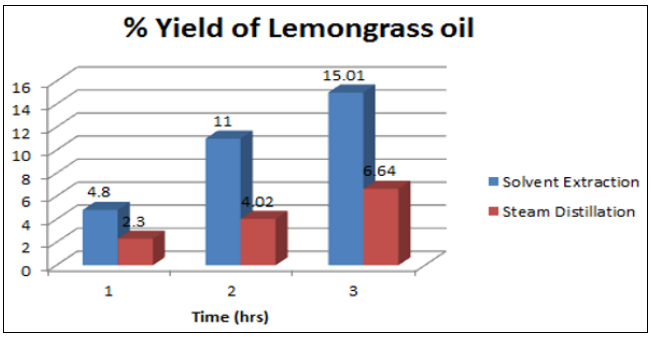


Fig 1.2: Show the Bar Chart Comparison of the Oil Yield of Lemongrass

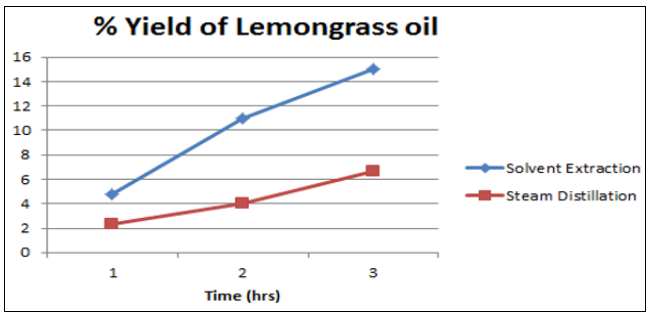


Fig 1.3: Show the Graph Comparison of Lemongrass Oil

t-Test: Two-Sample Assuming Unequal Variances

	Solvent Extraction	*Steam Distillation*
Mean	10.27	4.32
Variance	26.4607	4.7764
Observations	3	3
Hypothesized Mean D	0	
df	3	
t Stat	1.843920697	
P(T<=t) one-tail	0.081201586	
t Critical one-tail	3.182446305	
P(T<=t) two-tail	0.162403171	
t Critical two-tail	4.176534846	

Fig 1.4: Show the t-test analysis

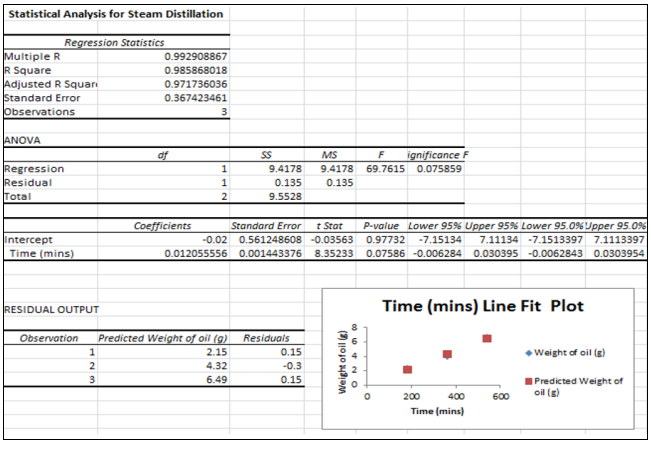
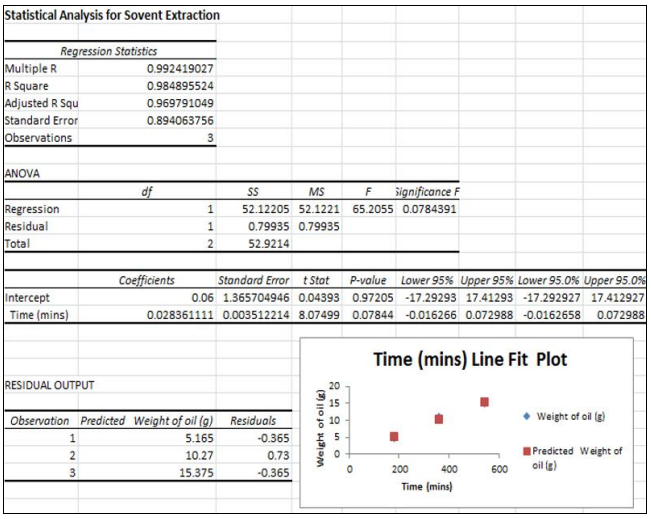


Fig 1.5: Show the Regression Analysis for Steam Distillation



Statistical Analysis for Sovent Extraction

Regression Statistics	
Multiple R	0.992419027
R Square	0.984895524
Adjusted R Squ	0.969791049
Standard Error	0.894063756
Observations	3

ANOVA

	df	SS	MS	F	Significance F
Regression	1	52.12205	52.1221	65.2055	0.0784391
Residual	1	0.79935	0.79935		
Total	2	52.9214			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	0.06	1.365704946	0.04393	0.97205	-17.29293	17.41293	-17.292927	17.412927
Time (mins)	0.028361111	0.003512214	8.07499	0.07844	-0.016266	0.072988	-0.0162658	0.072988

RESIDUAL OUTPUT

Observation	Predicted Weight of oil (g)	Residuals
1	5.165	-0.365
2	10.27	0.73
3	15.375	-0.365

Fig 1.6: Show the Regression Analysis for Solvent Extraction

T-test is used to show comparison, it is used to determine the statistical yield difference. From figure 4.6 the two-sample assuming unequal variance t stat is 1.8439, comparing it with t critical two-tail (4.1765), this show that t stat fall within the alternative hypothesis (do not reject region). P-value rule states that reject null hypothesis if p-value <= $\propto$ and do not reject null hypothesis if p-value >$\propto$. Since p-value is greater than **alpha**($\propto$= 0.025), from t test table P (T<=t) two-tail is 0.1624.

Regression analysis (r) measures the linear relationship between two quantitative variables with respect to direction and strength. While square regression analysis (r^2) is a measure of how close each data point fits to the regression line. From figure 4.7 and figure 4.8, the R square (0.9858 and 0.9848) shows how well the regression line predicts actual values.

Conclusion

The following conclusions were drawn from the investigation carried out.

1. The volume of lemongrass oil increase as extraction time increased and particle size decreased. The maximum yield was found at extraction time of 9 hours each using solvent extraction and Steam distillation respectively, the maximum yield is 5.00% for solvent extraction and 2.21% for steam distillation.
2. The yield of oil from soxhlet extraction was higher than what is achieved using Steam distillation. On the other hand steam distillation gives a volatile oil, while solvent extraction method gives concrete oil.
3. The oil had very low level of unsaturation and as such is a very good oil since it has low susceptibility to oxidation and rancidification. This makes it edible and fit for various uses.
4. The perfume produced using the oil had a strong, long-lasting pleasant smell. However, it itches the human skin minutes alter application and so should not be applied directly to the skin.

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