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Optimization of Phytochemical Screening Analysis of *Ocimum Gratissimum* Leaf Oil Extraction Process

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Abstract

Optimization of the process variables for the extraction of oil from *Ocimum gratissimum* (scent leaves) was studied. The effects of various process variables such as temperature, time, volume of solvent, particle size and their interaction on oil yield were investigated. A predictive model describing the oil yield in terms of process variables was derived from multiple regression analysis. Optimum yield of (54%) was predicted at extraction temperature of 50°C, extraction time of 40 min, leaf particle size of 150µm and 125ml volume of solvent but decreased with increase in leaf particle size. The extract was analysed to examine the physiochemical properties such as acid value, iodine value, peroxide value, viscosity, saponification value, specific gravity, moisture and ash contents using standard methods. Results revealed that the oil is edible and can find uses in food and pharmaceutical industries for spice and drug production respectively.

Introduction

Plants are basis of traditional medicine and have an impact on modern system of medicine [41].

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Many of the indigenous plants are very cheap, readily available in the rural areas and are used as spices [4, 8]. The upsurge in the prevalence of the side effects of many synthetic antimicrobial agents and incidence of multi-drug resistant bacteria has spurred scientist unto the research for plant based antimicrobial and curative potentials of some herbs/spices [5]. Essential oils are used in a wide variety of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, distilled alcoholic beverages (hard drinks) and insecticides. They contain phytochemicals which are bioactive substances derived solely from plants and are associated with the protection of human health against chronic disease which do not act alone but in combination of complex organic substances [13]. The knowledge of the chemical constituents of plant is desirable not only for the discovery of the therapeutic agents but also because such information is of value in disclosing new sources of economic material as tannins, gum, oil, precursors for the synthesis of complex substance [14]. *O. gratissimum* is one of those plants widely known and used for both medicinal and nutritional purposes. It belongs to the family of 'Labiatae'. It is a perennial plant widely distributed in the tropic of Asia and Africa, particularly Nigeria. The common names of the plants are scent leaves, Basil fever plant, tea bush; its vernacular name includes; Daidoya (Hausa), Nchuanwu (Igbo), Efinrin (Yoruba) among the major ethnic groups in Nigeria. The plant belongs to the family of Labiatae; it is woody at the base and has an average height of 1-3m. Its leave are broad and narrowly ovate, usually 5-13cm in length and 3-9cm wide. The plant is usually consumed by the Igbo's as a leafy vegetables and the nutritional importance of the plant centres on its usefulness as seasoning because of its aromatic and characteristic flavour [4].

It is traditionally used in the management of the baby's cord and wound surface as it is believed to keep it sterile [12]. The crushed leaf juice is used in the treatment of stomach pain; convulsion, catarrh and the oil from the leaves have been found to posses' antiseptics, antibacterial, anti-malaria, anti-diabetic and anti-fungal activities [11]. The plant is found to thrive well in regions 1500 m above sea level [10]. The method of propagation is mainly by stem cutting, which usually take 28 days to form roots. It requires well drain soil and full exposure to sunlight [7]. The onus of this study is to optimize various independent variable conditions for maximum yield of essential oil from *O. gratissimum*.

Design of experiment

Design of experiment (DOE) is a computer-enhanced systematic approach to

experimentation that considers all factors involved simultaneously toward optimization of process variables for the extraction of oil from *O. gratissimum*. DOE is concerned with the planning and conduct of experiments to statistical screening analyses of the resulting data so that valid and optimal conclusions will be drawn.

Materials and Methods

Materials

The leaves of *Ocimum gratissimum* were collected from home garden at Uke, Idemili North Local Government Area of Anambra state, Nigeria. Identification of the leaf was carried out by the Botany Department of University of Nigeria, Nsukka. The freshly collected leaves were washed and shade dried for two weeks.

Extraction of plant extracts

The extraction of plant extracts followed similar process adopted by [2, 4, 9].

FTIR analysis of plant extracts

The FTIR analysis of leaves extract was carried out as reported by Uzoh et al. [32] except for the use of Shimadzu FT-IR spectrophotometer model: IR affinity-1.5/ (NA 2137470136SI).

GC-MS analysis

GC-MS analysis was carried out on a mass spectrophotometer model No QP 2010 plus Shimadzu, Japan. The carrier gas used was Helium at a flow rate of 0.5m/min. 1µ sample injection volume. The inlet temperature was maintained at 240°C and then programmed to increase to 280°C. Total run time was 90min. The MS transfer time was maintained at a temperature of 200°C. The source temperature was maintained at 180°C. The peaks in the chromatogram were integrated and compared with the data base of spectra stored in the GC-MS library [32].

Qualitative analysis of the extracts

The solvent free extract obtained as above is then subjected to qualitative test for the identification of various plant constituent from the sample. Phytochemical characterization of genotypes is qualitative; data were collected on the presence or absence of the metabolites essential oils, antraquinones, base alkaloids, weakly base alkaloids, phenols and tannins, quaternary salts, flavonoids, and saponins of the plant.

Materials on which determinations are performed are shade-dried and converted to dust using a laboratory mill [35]. Thus, these processed materials are exposed 10 times to hexane for fat removal. After this procedure, materials are kept under hexane for 24 h, filtered, and left for 5 to 7 days to allow the residual hexane to evaporate. This processed material is covered with ethanol and maintained for 24 h. A series of several filtering is performed until the liquid material turned into hyaline aspect. Assays are conducted with this liquid to determine the presence or absence of each one of the above-mentioned metabolites.

For base and weakly base alkaloids, as well as for quaternary salts, the raw extract of ethanol evaporated until complete dryness received hydrochloric acid at 10%. Mixed material is passed through a separation funnel. The first separation is attained with chloroform. To the aqueous phase that remained in the funnel, a portion of ammonium hydroxide is added until it reached a basic pH. With this test, we are able to determine the type of alkaloids present based on and then it is washed again with chloroform to obtain the second phase. The third phase had been left in the funnel. Using a capillary tube, a few drops of each one of the three phases are placed on plaques of silica gel and sprayed with Dragendorf compound. Assays in which the phase responded positively to the presence of alkaloids formed a black spot which phase (first, second, or third) reacted to the Dragendorf compound. These results are confirmed by treating the three phases with Meyer's reactive, resulting in a white precipitate with a small amount of the raw extract [39].

Quantitative tests for phytochemicals

Phytochemical screening was carried out on *O. gratissimum* using standard procedures to establish the constituents; Terpenoids, Flavonoid, Tannins, Alkaloids, Phenols, Saponins and Steroids as described by Sofowara [35], Okwu and Okwu [36], Ladipo et al. [12], Harbone [20], Marcano and Hasenawa [22], Hill [40] and Edeoga et al. [39].

Physical properties of extracts

Determination of boiling points

1g of the extracted oil was placed in a test tube and a thermometer was inserted in it for some minutes and was placed on a heating mantle, it was observed that the oil in the beaker began to circulate leading to boiling of the oil. The temperature at that point was noted as the boiling point of the oil.

Determination of specific gravity

A clean dry density bottle of 25ml capacity was weighed in an electronic balance w_1 and then filled with oil and weighed w_2. The oil was substituted with water after washing, rinsing and weighed to give w_3.

$$S_G = \frac{w_2 - w_1}{w_3} \tag{1}$$

where S_G is the specific gravity, w_1 is the weight of density bottle, w_2 is the weight of density bottle and oil and w_3 is the weight of the density bottle and water [9].

Determination of melting point

4g of the oil was filled in a test tube and allowed to solidify. The oil was brought out and held in a clamp. The thermometer was inserted immediately it started defrosting and the change in temperature was critically observed. The temperature at which there was a sudden temperature variation was taken and noted as the boiling point of the oil.

Determination of acid value

2g of the oil extracted at 25°C was properly weighed into a beaker and dissolved with 0.5ml of chloroform. The solution was thoroughly dissolved and titrated with 0.1M KOH using 1ml of phenolphthalein indicator. The end point was reached when a pink colour was seen which persisted for 30seconds.

$$A_v = \frac{V \times M \times 56.1}{m} \tag{2}$$

where A_v is acid value, V is the volume of KOH; M is the morality of KOH, m is the mass of test portion and 56.1 is the molar mass of KOH.

Determination of Saponification value

2g of the oil extracted at 25°C was weighed into a conical flask. 25 ml of alcoholic KOH was added to the test portion using a pipette. A reflux condenser was connected to the flask and place on a steam bath, then boiled gently at temperature of 35°C and stirred vigorously for 1hr. 1 ml of phenolphthalein was added to the hot solution and titrated with 0.5M HCl until a purple colour of the indicator changed to yellow. The blank test was carried out following the above procedures but the test portion was omitted.

$$S_PV = \frac{(v_1 - v_2) \times M \times 56.1}{m} \tag{3}$$

where, S_PV is the saponification value, v_1 is the volume of HCl used for the blank test; v_2 is the volume of HCl used for determination, M is the morality of HCl, m is the mass of test portion and 56.1 is the molar mass of KOH solution.

Determination of iodine value

2g of the oil extracted at 30°C was weighed into the titration bottle, 20ml of chloroform was added to the flask and 10ml of glacial acetic acid was added to the clear solution in the burette. 100ml of the iodine mono chloride was also added to the solution and was kept in the dark cupboard for 2hrs. The solution was stirred vigorously and then potassium iodide solution was added to the solution and stirred vigorously (addition of potassium iodide is to convert the unused reagent to iodine). The solution was then titrated with a standard solution of 0.1M sodium thiosulphate to a clear colourless endpoint. The same procedure was used for the blank test. The experiment was repeated with other oil extracts. The iodine value is given by the expression:

$$I_v = \frac{(v_1 - v_2) \times M \times 12.69}{m} \tag{4}$$

where, I_v is the Saponification value, v_1 is the volume of sodium thiosulphate solution used for the blank test; v_2 is the volume of thiosulphate solution used for determination, M is the molarities of sodium thiosulphate , m is the mass of test portion and 12.69 is the molar mass of iodine solution

Process design matrix

Preliminary data analysis conducted using steepest ascent method shows that a curvature effect is possible. In view of curvature, a reduced order quadratic model (ROQM) was fitted over the resulting data as suggested in Eq. (4)

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i<} \sum_{j=2}^{k} \beta_{ij} X_i X_j + \sum_{i=1} \sum_{j=1}^{k} \delta_{ij} X_i X_j + \varepsilon. \tag{5}$$

Equation (5) serves as the global predictive equation from which specific solution may be derived. The determination of the unknown coefficients of β_0, β_i, β_{ij} and δ_{ij} is accomplished via regression analysis implemented on the statistical analysis software

Design-Expert Version 9.1.7.1 trial from the Stat-Ease Inc. using the data recorded from the investigation.

The determination of unknown coefficient of Eq. (4) applies the design matrix of Table 1 formulated by judicious transformation of the actual values of the four control variables at various levels over which the experiments were executed to their coded equivalents using −1 and +1 notations to designate low and high level factor setting and '±α' and '0' for axial and centre points, respectively. The coded values of the independent variables for the design of the experiment for *O. gratissimum* leave oil extraction process are given in Table 1. For statistical analysis, the variables $X_i (i = 1, 2, ..., 4)$ were coded A, B, C and D. The data given in Table 1 were used to formulate a global design matrix of Table 1 below from which further analyses were derived. *Y* is the response (oil yield) across the various experimental runs Equation (4) was fitted to the experimental data presented in Table 1 to obtain the final predictive equation for their action progress in terms of the coded variables.

Table 1. The experimental range and level of the independent variables for scent leaf extraction

Independent variable	Range and level				
	−α	−1	0	1	+α
Leaf particle size (μm) (A)	150	300	600	750	1000
Temperature (°C) (B)	20	30	40	50	60
Volume of solvent (ml) (C)	50	75	100	125	150
Time (min) (D)	10	20	30	40	60

Results and Discussion

The result of phytochemical screening of *Ocimum gratissimum*, Table 2 shows that the plant leaves contains tannins, saponins, flavonoids, terpenoids, alkaloids, plobatannins and glycosides. Further analysis of the phytochemicals constituents with GC-MS ascertains that *Ocimum gratissimum* contains all the necessary phytochemical constituents. These metabolites are known to have varied pharmacological actions in man and animals, the presence of these metabolites suggest great potentials of the plants

as a source of useful phytomedicines. The phytochemicals are naturally occurring chemicals in plants which serve as medicinal for the protection of human disease; the phytochemical are non nutritive plants chemical that have protection or disease preventive properties [34]. Alkaloids are also considered as nitrogenous bases that occur in plants, many of them have marked physiological effects on humans. Some alkaloids used as medicine are morphine, caffeine and coffee; in which caffeine in tea and coffee is alkaloids that stimulate the nervous system [33]. The presence of alkaloids suggests that it has potential antimicrobial activity on microorganisms. Some plants that posse alkaloids are known for decreasing blood pressure and balancing the nervous system in case of mental illness. Alkaloids are known to posses' anti-malaria property; hence the plants may be a good source of anti-malaria for which it is traditionally used. Flavonoids are polyphenolic compound that contribute to many other colours found in nature particularly the yellow and orange of petal, they have been reported to have antiviral and antiallegic activities. Presence of flavonoids might be responsible for its use as anti-inflammatory effects on both acute and chronic inflammation [34-38]. The presence of saponins serves as potential activity of an antimicrobial agent. The presence serves as an indicator towards possible antibacterial activity. Saponins are a class of natural products involves and can be used to enhance penetration of micro molecules such as protein through cell membrane

Table 2. Phytochemical constituents.

Constituents Result	GC-MS
Tannins	+
Saponins	+
Flavonoids	+
Terpenoids	+
Alkaloids	+
Steroid	+
Glycosides	+
Plobatannis	+

Key : - = Absent, + = Present

Table 3. Central composite rotatable design matrix for scent leaf oil extraction process.

Runs	Independent variables				Responses
	A(μm)	B(°C)	C (ml)	D (min)	Y(%)
1	−1.00	1.00	1.00	1.00	31.79
2	1.00	1.00	−1.00	1.00	17.67
3	0.00	0.00	0.00	0.00	31.67
4	0.00	0.00	2.00	0.00	43.56
5	1.00	−1.00	−1.00	1.00	20.14
6	0.00	0.00	0.00	0.00	31.62
7	1.00	1.00	−1.00	−1.00	15.43
8	0.00	−2.00	0.00	0.00	21.54
9	1.00	−1.00	−1.00	−1.00	17.75
10	−1.00	1.00	−1.00	−1.00	32.76
11	0.00	0.00	−2.00	0.00	20.81
12	1.00	−1.00	1.00	−1.00	19.66
13	0.00	0.00	0.00	0.00	31.65
14	−1.00	−1.00	−1.00	1.00	20.55
15	1.00	−1.00	1.00	1.00	22.68

3-D response surface plots for the optimization process

The 3-D response surface plots are graphical representations of the interactive effects of any two variables. The nature of the response surface curves shows the interaction between the variables. An elliptical shape of the curve indicated good interaction of the two variables and circular shape indicated no interaction between the variables. The 3-D response surface plots shown in Figures 1-6 for the chosen model equation showed the relationship between the independent and the dependent variables. From Figure 6, the response surface indicated that the percentage yield of oil increases as temperature and solvent composition increases to optimum condition while further increase leads to decrease of percentage yield of oil. In addition, there was mutual

interaction between the temperature and solvent composition. The highest percentage oil yield was obtained when 153ml of solvent was used. This is in accordance with the result obtained by Topallar and Gecgel [30], Gunawan and Suhendra [19] that studied kinetics and thermodynamics of oil extraction from olive cake. They reported that the positive effect of volume of solvent on oil yield was as a result of increase in the concentration driving force as volume of solvent increases. It was also as a result of increased washing of the oil extracted away from the particle surface by the solvent as a result of increased volume. The increase in oil yield became less significant at 125ml because 125ml hexane was sufficient to bring the oil solute to equilibrium. Similarly, the oil yield increased as the temperature increased from 30 to 50°C. The highest oil yield was obtained at 50°C.

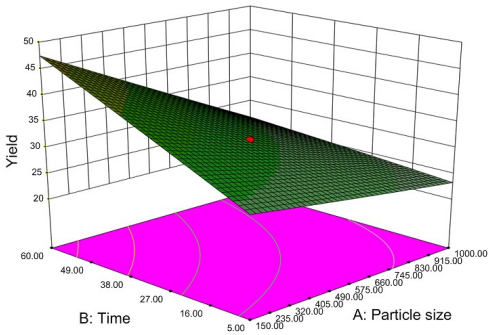

Figure 1. Response surface plot showing the 3D effect of time and particle size and their interaction effect on the yield of scent leaf oil.

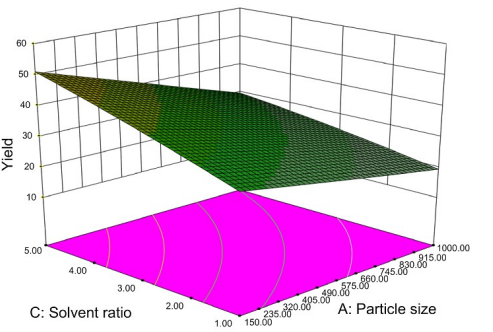

Figure 2. Response surface plot showing the 3D effect of solvent ratio, particle size and their interaction effect on the yield of scent leaf oil.

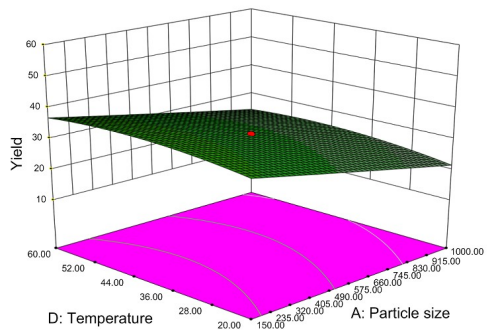

Figure 3. Response surface plot showing the 3D effect of temperature and particle size and their interaction effect on the yield of scent leaf oil.

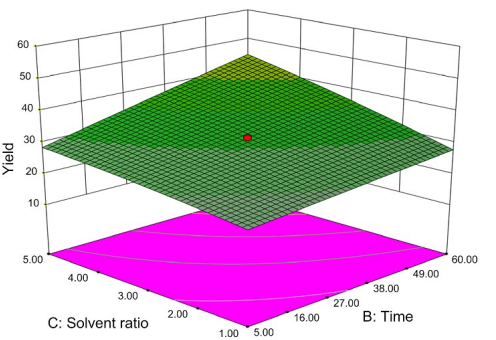

Figure 4. Response surface plot showing the 3D effect of solvent ratio, time and their interaction effect on the yield of scent leaf oil.

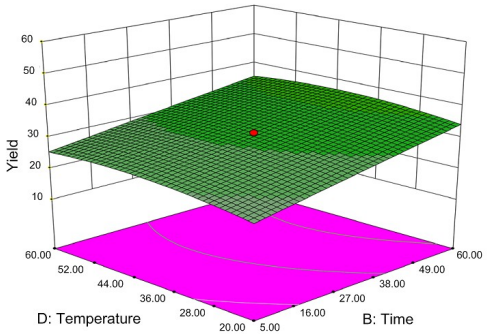

Figure 5. Response surface plot showing the 3D effect of temperature, time and their interaction effect on the yield of scent leaf oil.

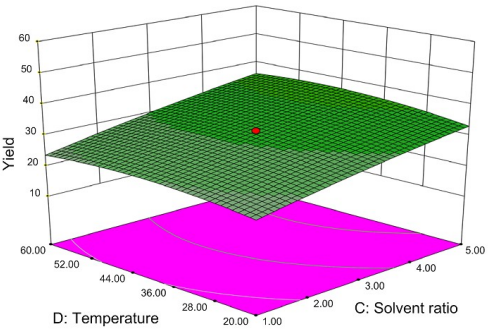

Figure 6. Response surface plot showing the 3D effect of temperature and solvent ratio and their interaction effect on the leaf oil.

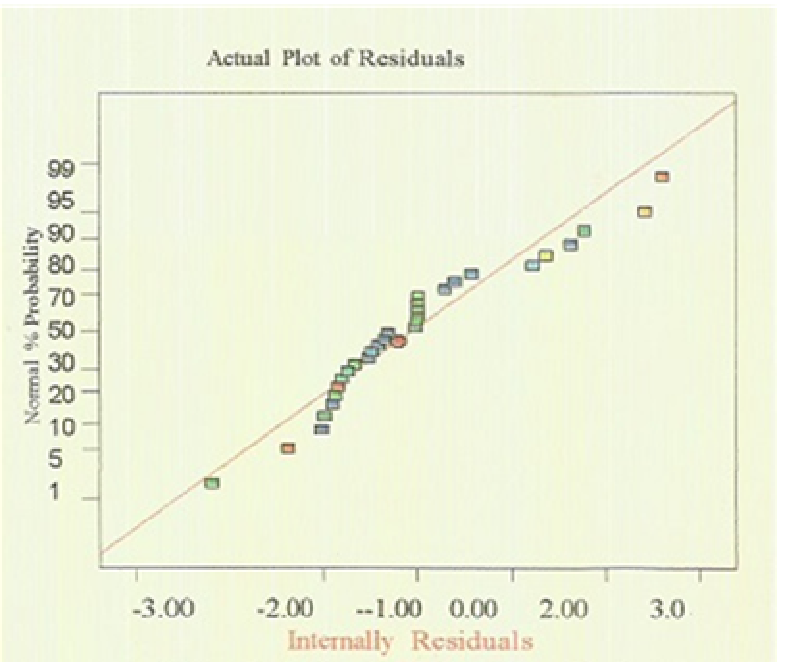

Figure 7. Actual plots of residuals.

The positive effect of temperature on oil yield is as a result of rupturing of oil cell walls which now creates a void which serves as migratory space for the contents of the oil bearing cells [6, 24]. Temperature influences oil yield and higher extraction is achieved by increasing the temperature which lowers the viscosity of the released oil

from the intact cells and draws out moisture. Figures 1-3 display the responses for the interactive factors; time (x2) against seed particle size (x1), solvent ratio (x3) against seed particle size (x1), and temperature (x4) versus particle size (x1), respectively. The 3-D response surface plots shown in Figures 1, 3 and 4 show minimal drop in percentage oil yield when seed particles size increases; even at highest setting of temperature of (60°C), time of (60 min), solvent ratio of (5:0).The negative effect of seed particle size on oil yield could be at tribute to the fact that smaller particles have larger amount of surface area coupled with increased number of ruptured cells resulting in a high oil concentration at the particle surface and low or little diffusion into the particles surface. Narayana et al. [23], Sayyar et al. [27], while investigating the extraction of oil from Jatropha seed, suggested also that large particles have smaller amount of surface areas and are more resistant to intrusion of solvent and oil diffusion. Therefore, small amount of oil will be carried from inside the large particles to the surrounding solution. The quadratic effect of temperature (D) is visibly evident from the smooth curve in the response surface plots Figures 2 and 6. Dragon and Reyes [18], reported (46%) oil yield at extraction time of 120 min for scent leaf oil using solvent extraction method while the current research recorded (49.50%) oil yield at extraction time of 60min. Overall, given the long operational time, the earlier report may not be economically advantageous in terms of energy savings.

The normal plot of residuals (Figure 7) was used to check whether the points will follow a straight line in which we concluded that the residuals follow normal distribution. Hence from Figure 7, it is seen that the points were closely distributed to the straight line of the plot. It confirms the good relationship between the experimental values and the predicted values of the response. Though some small scatter like an "S" shape is always expected, these plots equally confirm that the selected model was adequate in predicting the response variables in the experimental values.

GC-MS analysis of O. gratissimum leaf

The fatty acid composition of scent leave oil was analysed by gas chromatography mass-spectrometry (GC–MS). Table 4 shows the fatty acids present in the scent leaf oil. GC–MS analysis of the oil showed abundance of palmitoleate (31.94%wt) and arachidic acid (17.16%wt). Thermostat abundant unsaturated and saturated fatty acids were palmitoleate (31.94 %wt) and methyl stearate (14.22%wt), respectively. The oil contains (50.86%) saturated fatty acid and (49.10%) unsaturated fatty acid. The results for the

physico-chemical properties of the extracted oil are presented in Table 4. The physico-chemical analysis of the oil indicated physical state of the oil to be liquid and amber yellow at room temperature. The oil content of *O. gratissimum* was found to be (54.6 %) wt. The oil contents are significant and compared favourably with leaf oil of other plants such as olive leaf (49.86%wt), [29, 6]. On the basis of the oil contents, O. *gratissimum* would be highly suitable for consumption.

Table 4. Fatty acid composition of scent leave oil.

Carbon Molecules	Name	%wt
C_{10}	Capric acid	1.27
C_{12}	Lauric acid	6.00
$C_{20:2}$	Arachidic acid	17.16
C_{14}	Myristste	4.59
$C_{16:1}$	Palmitoleate	31.94
C_{17}	Magaric acid	4.84
C_{18}	Methyl stearate	14.22
C_{25}	Pentacosylic acid	3.70
C_{30}	Melissic acid	5.75

A very negligible or no risk of fire outbreak in case of accident will be obtained when used as biodiesel or spills. A value of 1.441 was obtained for the refractive index. The refractive index value obtained falls within the range (1.447-1.490) reported for some other leaf oils (1.480 for Telfairia occidentalis, 1.468 for Jatropha curcas, 1.47 for soybean oil and 1.47 for corn oil) which have myriad industrial applications. Ejikeme et al. [7] reported that the specific gravity of scent leaf oil was found to be 0.892 at 25 OC and this value is in the range found for other common oils. The SG value is also within the range (0.860-0.900) stipulated by EN14214 for bio-diesel [21]. Iodine value of 31.09 ml eqg-1was obtained.

Characteristics of scent leaf oil

Iodine value measures the degree of unsaturation of scent leaf oil. Oils with iodine value above 135 are classified as drying oil, those with iodine value 110-130 are classified as semi-drying oil and those with iodine value below 90 are non-drying oils. The iodine value is also consistent with the corresponding total saturation of fatty acids (50.86%) listed on Table 5, thus affirming the oil is largely consisting of saturated fatty acids and is non-drying.

Peroxide value of 9mleqg^{-1} was obtained for the oil. Peroxide value is affected by alot of conditions which include oxidation by oxygen, extraction methods, and storage. The low peroxide value suggests that scent leave oil is stable to oxidative degradation caused by over exposure to oxygen, heating and improper storage [17, 28]. Saponification value of 32.258 mleqg^{-1} was obtained for the sent leaf oil. The saponification value is very low when compared with the values for common oils: palm oil (196-205), groundnut oil (188-196), and corn oil (187-196) [26]. The low saponification value is as a result of the abundant long chain fatty acids (found in the oil) which have a relatively fewer number of carboxylic functional groups per unit mass of the oil as compared to short chain fatty acids. Thus the oil is not suitable to be used for soap production. An acid value of 0.0336 mgKOH g^{-1} gave an indication of the amount of FFA present in the oil at the time of the test. The low acid value is an indication of good non-degraded state of the oil and is within limits for industrial useful oils. The FFA concentration of the oil was low (0.0168) which was consistent with low acid value observed. The low FFA also suggests low levels of hydrolytic and lipolytic activities in the oils.

Table 5. Physico-chemical properties of scent leaf oil.

Physico-chemical parameters	Values	Units
Specific gravity	0.89	-
Acid value	3.37	%
Viscosity	3.63	pa.s
Peroxide value	9	mleqg^{-1}
Saponification value	32.26	mleqg^{-1}
Iodine value	31.09	mleqg^{-1}
Moisture content	7.5	%
Flash point	331.54	°C
Smoke point	176	°C
Refractive index	1.44	-
Fire point	386.12	°C
Oil content	54.6	%wt
Ash content	5	%
Free fatty acid	1.68	%

FTIR analysis of O. gratissimum oil

The moisture content of the oil (7.5%) was very high; the chemical functional organization of extracted scent leaf oil was investigated by FTIR as shown in Figure 8. In the spectrum of *O. gratissimum* oil, 3,498.02 cm^{-1} correspond to the hydroxyl group (O–H) of the unsaturated fatty acid in the oil. The carboxyl group (C=O) is indicated at 1,646.3cm^{-1}. The straight chain of –CH– stretch in aliphatic compound is found at the band 2,924.18 cm^{-1}. Alkene group (CH=CH) is attributed to the band of 3,206.78 cm.

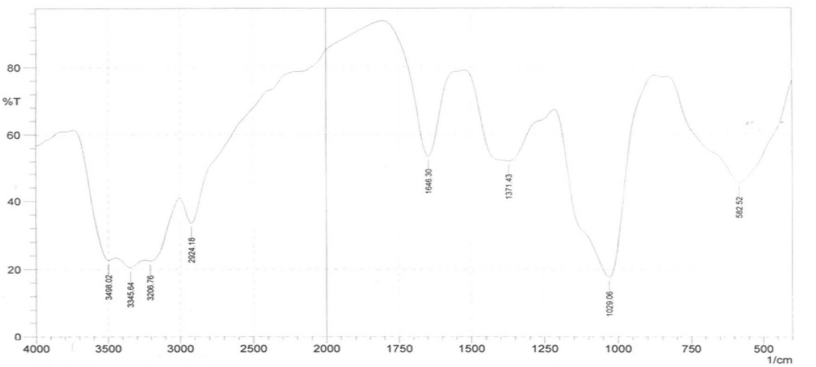

Figure 8. FTIR analysis of *Ocimum gratissimum* oil.

Kinetic study on extraction of scent leave oil

Linearization of the Arrhenius law as shown below gives the values of the activation energy (E) and the temperature independent factor (A) from ln(k) against 1/T plot:

$$\ln(k) = \ln(A) + (-E/R)1/T\,.$$

The plot of ln(k) against 1/T gives ln(A) as the slope and (–E/R) as the intercept. A plot of ln(dY/dt) versus lnY was found to be linear. A second order kinetics was obtained from the slope of the straight line as shown in Figure 9. The reaction rate constant was determined from the intercept as 1.26×10^{11} $(dm^3\ mol^{-1})^3 S^{-1}$.

The positive value of enthalpy change indicates that the process is endothermic and requires energy during process.

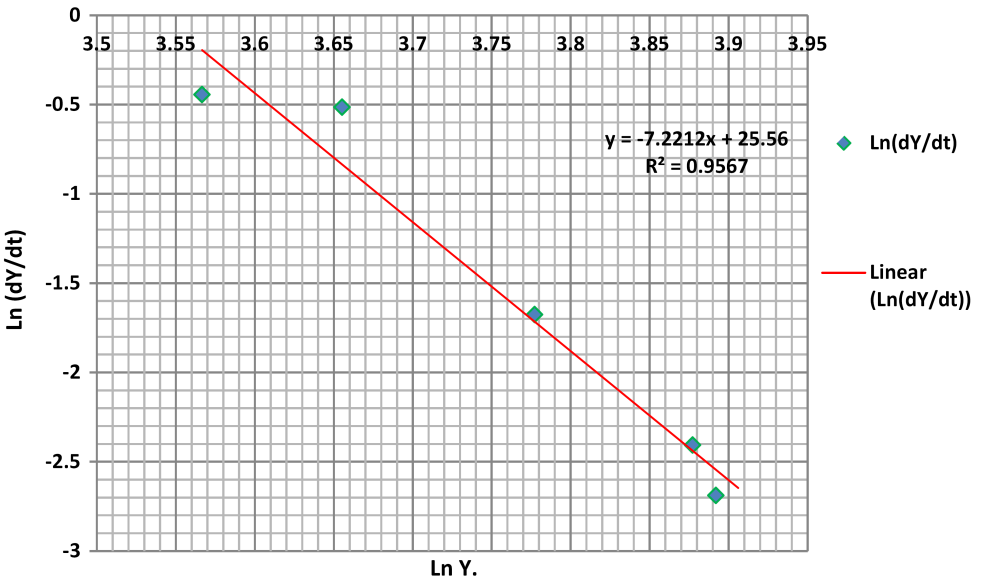

Figure 9. A plot of ln(dY/dt) versus ln Y.

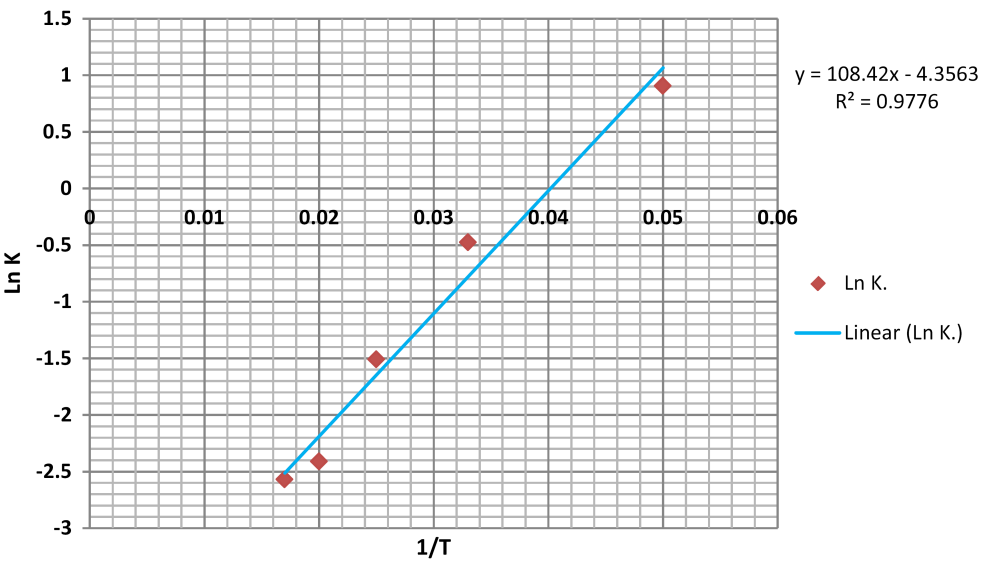

Figure 10. A plot of ln(K) versus 1/T.

Conclusion

This study has clearly demonstrated the applicability of RSM in selecting extraction conditions for scent leaf oil. The approach has not only resulted in the maximum oil

yield through solvent extraction, but has also guaranteed the fulfillment of the properties requirements of the bioactive nutrients in the oil. The optimum values for yield showed that it is an economic source of oil; the low saponification value means that it is not a good ingredient for soap making. The oil is very saturated and can hardly be used for paint making but when combined with other substances it can be used as a finishing agent. Time, particle size, temperature, and quantity of solvent have numerous effects on the yield of oil. The validation experiments and their accompany quality characteristics were not significantly different from the simulated values at $P < 0.05$. From spectroscopic results, it can be concluded that scent leaf oil can be used as a source of consumable spices.

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