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Research Paper

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 150 µm and 125 ml volume of solvent but decreased with increase in leaf particle size. The extract was analyzed 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 be used in food and pharmaceutical industries for spice and drug production respectively.

Key words: Optimization, *Ocimum gratissimum*, response, surface methodology, regression analysis.

INTRODUCTION

Plants are basis of traditional medicine and have an impact on modern system of medicine (Elujoba, 2000). Many of the indigenous plants are very cheap, readily available in the rural areas and are used as spices (Alexander, 2016; Ekhaise et al., 2010). The upsurge in the prevalence of the side effects of many synthetic antimicrobial agents and incidence of multi-drug resistant bacteria has spurred scientist into the research for plant based antimicrobial and curative potentials of some herbs/spices (Abudullahi, 2012).

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 phyto-chemicals 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 (Okereke et al., 2017).

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 materials as tannins, gum, oil and precursors for the synthesis of complex substance (Oladosu et al., 2017).

Ocimum gratissimum is one of those plants widely known and used for both medicinal and nutritional purposes. The plant belongs to the family of 'Labiatae. It is a perennial plant that is widely distributed in the tropic of Asia and Africa, particularly Nigeria. The common names of the plants are scent leaves, Basil fever plant and tea bush; its vernacular name includes: Daidoya (Hausa), Nchuanwu (igbo) and Efinrin (Yoruba) among the major ethnic groups in Nigeria. *O. gratissimum* is woody at the base and has an average height of 1 to 3 m. Its leaves are broad and narrowly ovate, usually 5 to 13 cm in length and 3 to 9 cm wide. The plant is usually consumed by the Igbo's as a leafy vegetable and the nutritional importance of the plant centres on its usefulness as seasoning because of its aromatic and characteristic flavour (Alexander, 2016).

O. gratissimum is traditionally used in the management of the baby's cord and wound surface as it is believed to keep

it sterile (Ladipo et al., 2007). 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 properties (Lexa et al., 2007). The plant is found to thrive well in regions 1500 m above sea level (Prabhu et al., 2009). 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 (Ejikeme et al., 2010).

The onus of this study is to 0ptimize 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 towards optimization of process variables for the extraction of oil from *O. gratissimum*. The design of the experiment is concerned with planning and conducting of experiments to statistical screening analyses of the resulting data to ensure that valid and optimal conclusions are drawn.

MATERIALS AND METHODS

The leaves of *O. 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 Alexander (2016), Anadebe et al. (2017) and Ezeugo et al. (2018).

FTIR analysis of plant extracts

The FTIR analysis of leaves extract was carried out as reported by Uzoh et al. (2013) 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 0.5 m/min and 1 µ sample injection volume. The inlet temperature was maintained at 240°C and then programmed to increase to 280°C; total run time was 90 min. The MS transfer time was maintained at a temperature of 200°C, while 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 (Uzoh et al., 2013).

Qualitative analysis of the extracts

The solvent free extract obtained as earlier mentioned was then subjected to qualitative test for the identification of various plant constituents from the sample. Phytochemical characterization of genotypes is qualitative; data were collected in 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 were performed are shade-dried and converted to dust using a laboratory mill (Sofowora, 1993). Thus, these processed materials were ten times exposed to hexane for fat removal. After this procedure, materials were kept under hexane for 24 h, filtered, and left for 5 to 7 days to allow the residual hexane to evaporate. This processed material was then covered with ethanol and maintained for 24 h. A series of several filtering was performed until the liquid material turned into hyaline aspect. Assays were conducted with this liquid to determine the presence or absence of each of the aforementioned 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 was passed through a separation funnel. The first separation was attained with chloroform. To the aqueous phase that remained in the funnel, a portion of ammonium hydroxide was added until it reached a basic pH. With this test, we were able to determine the type of alkaloids present and was thereafter 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 were 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 (Edoja et al., 2005).

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 (1993), Okwu and Okwu (2005), Ladipo (2010), Harbone (1898), Marcano and Hasenawa (1991), Hill (1952) and Edeoja et al. (2005).

Physical properties of extracts

Determination of boiling points

1 g of the extracted oil was placed in a test tube and a thermometer inserted in it for some minutes and thereafter 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 25 ml 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.

$$S_G = \frac{\text{Mass of substance}}{\text{Mass of equal volume of water}} \quad (1)$$

$$S_G = \frac{w_2 - w_1}{w_3} \quad (2)$$

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 (Ezeugo et al., 2018).

Determination of melting point

4 g 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 immediately inserted and the solid started defrosting; 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

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

$$A_v = \frac{V \times M \times 56.1}{m} \quad (3)$$

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

2 g 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 placed on a steam bath, boiled gently at temperature of 35°C and stirred vigorously for 1 h. 1 ml of phenolphthalein was then added to the hot solution and titrated with 0.5 M HCl until a purple colour of the indicator changed to yellow. The blank test was carried out following the aforementioned procedures but the test portion was omitted.

$$S_PV = \frac{(v_1 - v_2)\ M \times 56.1}{m} \quad (4)$$

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

2 g of the oil extracted at 30°C was weighed into the titration bottle, 20 ml of chloroform was added to the flask and 10 ml of glacial acetic acid was added to the clear solution in the burette. 100 ml of the iodine mono chloride was also added to the solution and kept in the dark cupboard for 2 h. The solution was vigorously stirred and the potassium iodide solution added to the solution and also stirred vigorously (addition of potassium iodide is to convert the unused reagent to iodine). The solution was then titrated with a standard solution of 0.1 M 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:

$$I_v = \frac{(v_1 - v_2) \times M \times 12.69}{m} \quad (5)$$

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

Independent variable	Range and level				
	-α	-1	0	1	+x
Leaf particle size (**l** m)	150	300	600	750	1,000
Temperature (B)	20	30	40	50	60
Volume of solvent (ml) (C)	50	75	100	125	150
Time (min) (D)	10	20	30	40	60

Table 2: Phytochemical constituents.

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

Key: - = Absent, + = present.

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 Equation 5.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i<} \sum_{j=2}^{k} \beta_{ij} X_i X_j + \sum_{i=1} \sum_{j=1}^{k} \delta_{ij} X_i X_j + \varepsilon \quad (6)$$

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 through 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 Equation (6) 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 '± a' and '0' for axial and centre points, respectively. Table 2 shows the coded values of the independent variables for the design of the experiment for *O. gratissimum* leaf oil extraction process.

For statistical analysis, the variables Xjði¼1; 2;. ..; 4Þ were coded A, B, C and D. The data given in Table 1 was used to formulate a global design matrix of Table 2 from which further analyses were derived. Y is the response (oil yield) across the various experimental runs. Equation (5) 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.

RESULTS AND DISCUSSION

The result of phytochemical screening of *O. gratissimum* showed that the plant leaves contains tannins, flavonoids, terpenoids alkaloids plobatannins, tannins saponins, steroids and glycosides (Table 2). Further analysis of the phytochemicals constituents with GC-MS ascertains that *O. 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 suggests great potentials of the plants as a source of useful phytomedicines. The phytochemicals are naturally occurring chemicals in plants which serve as medicine for the protection of human disease; the phytochemical are non-nutritive plants chemicals that have protection or disease preventive properties (Cheng et al., 2002).

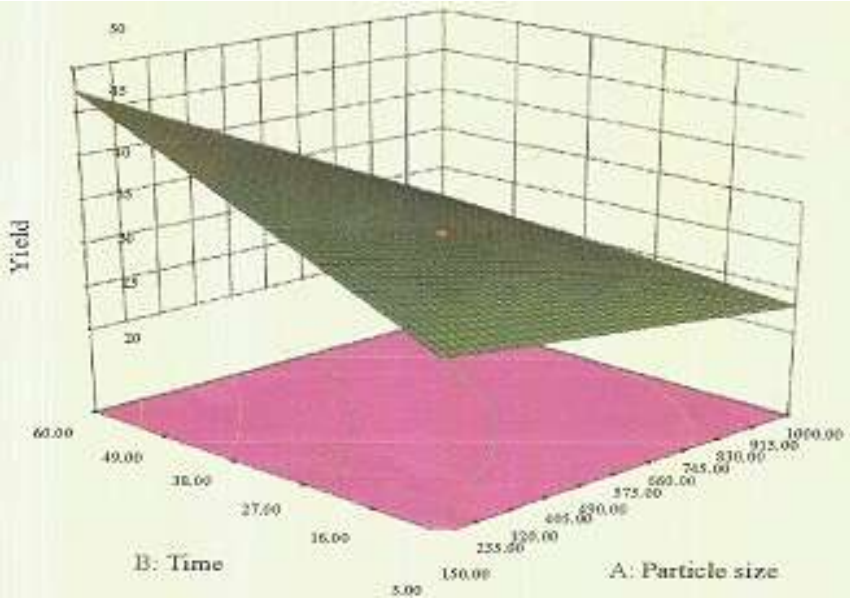

Figure 1: Response surface plot showing the 3D effect of time and particle size and their interaction effect on the yield of scent leaf oil.

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 are alkaloids that stimulate the nervous system (Stanley et al., 2007). The presence of alkaloids suggests that it has potential antimicrobial activity on micro-organisms. Some plants that possess alkaloids are known for decreasing blood pressure and balancing the nervous system in the case of mental illness. Alkaloids are known to posses' anti-malaria property; hence, the plant may be a good source of anti-malaria for which it is traditionally used (Stanley et al., 2007).

Flavonoids are polyphenolic compounds that contribute to many other colours found in nature particularly the yellow and orange of petal; they have been reported to have antiviral and anti-allergic properties. The presence of flavonoids might be responsible for its use as anti-inflammatory effects on both acute and chronic inflammation (Boham and Kocipai, 1994). 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 and can be used to enhance penetration of micro molecules such as protein through cell membrane.

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 to 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 153 ml of solvent was used. This is in accordance with the result obtained by Topallar et al. (2000) and Gunawan et al. (2008) 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 125 ml because 125 ml 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.

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 (Nayak et al., 2010; Ebelewe et al., 2010). 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 to 3 display the responses for the

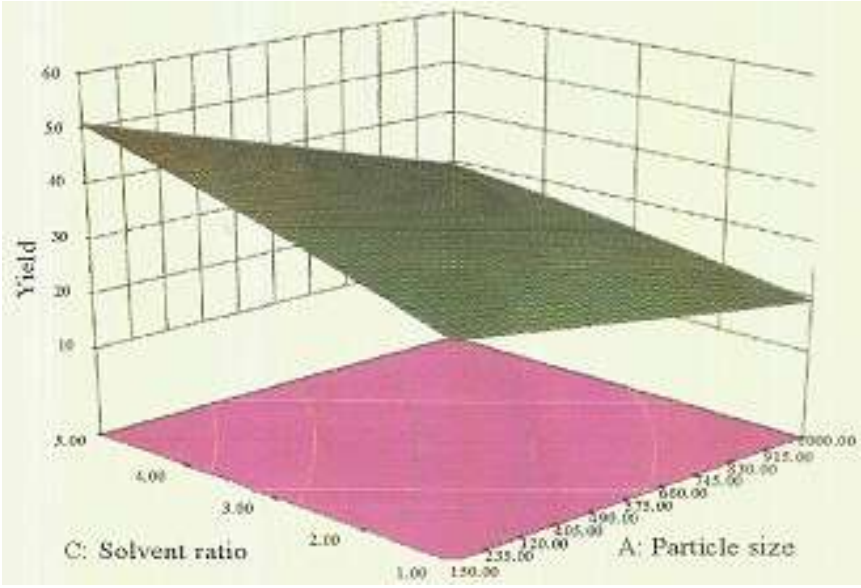

Figure 2: Response surface plot showing the 3D effect of solvent ratio, particle size and their interaction effect on the yield of scent leaf oil.

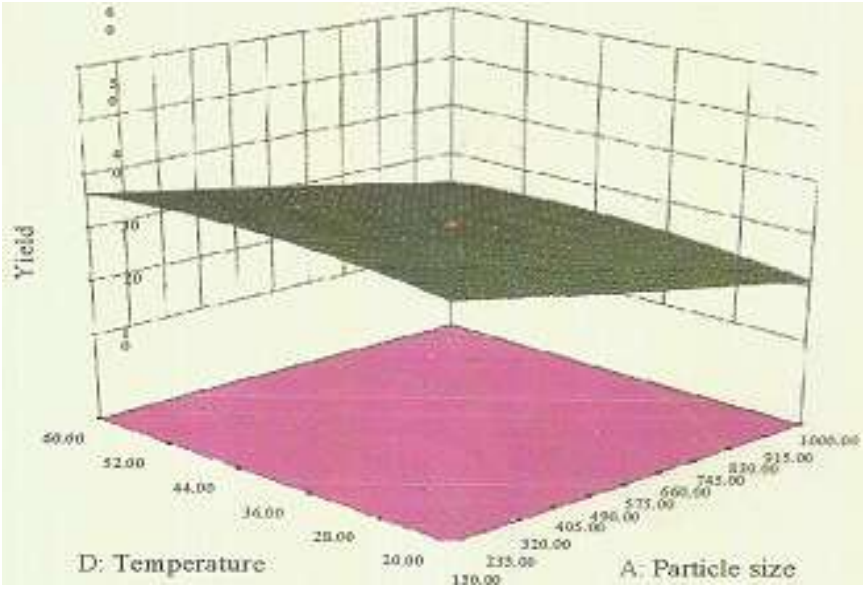

Figure 3: Response surface plot showing the 3D effect of temperature and particle size and their interaction effect on the yield of scent leaf oil.

interactive factors; time (x_2) against seed particle size (x_1), solvent ratio (x_3) against seed particle size (x_1) and temperature (x_4) versus particle size (x_1), 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 attributed 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. (2011) and Sayyar et al. (2009), 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

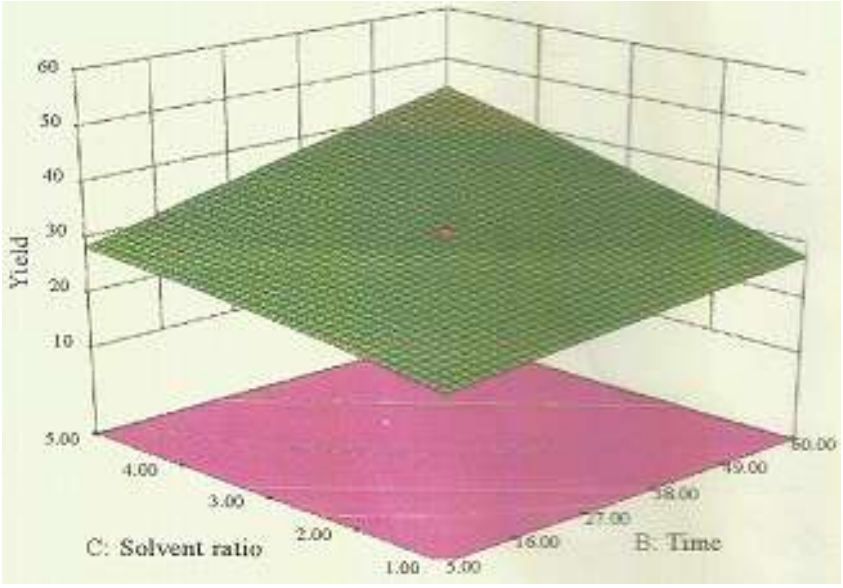

Figure 4: Response surface plot showing the 3D effect of solvent ratio, time and their interaction effect on the yield of scent leaf oil.

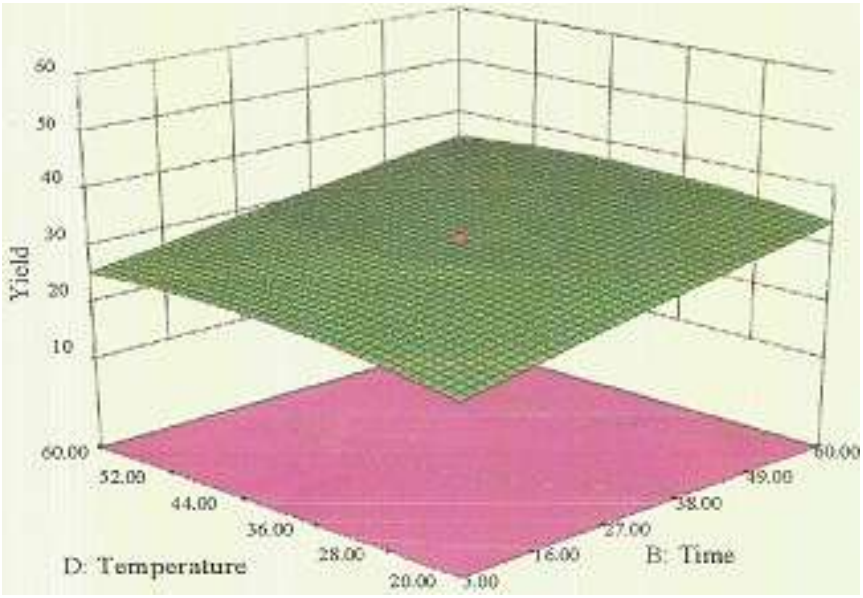

Figure 5: Response surface plot showing the 3D effect of temperature, time and their interaction effect on the yield of scent leaf oil.

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 et al. (2008), 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 60 min. 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 the 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;

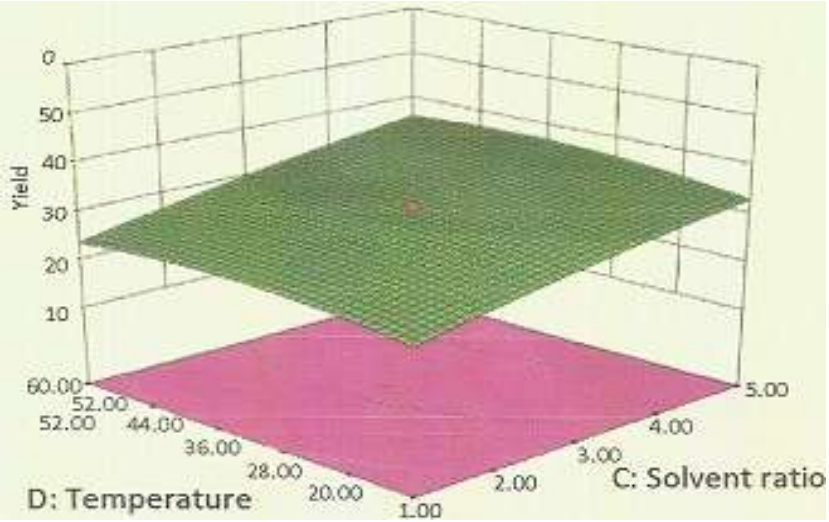

Figure 6: Response surface plot showing the 3D effect of temperature and solvent ratio and their interaction effect on the leaf oil.

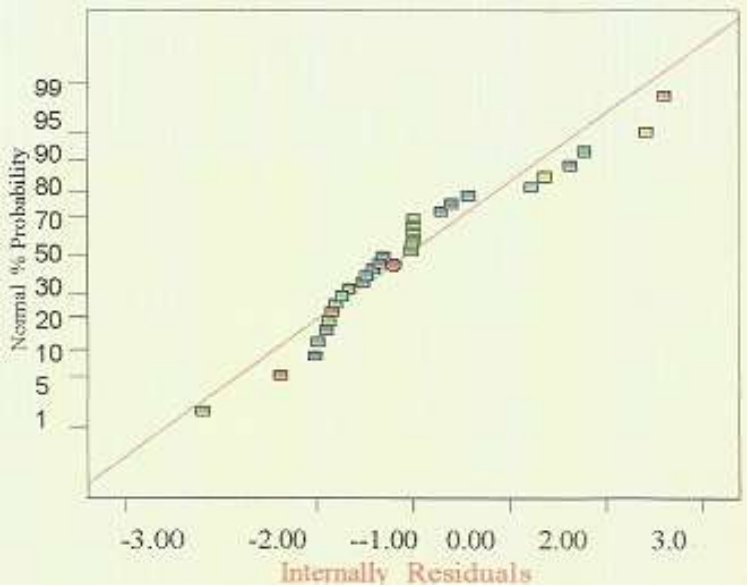

Figure 7: Actual plots of residuals.

these plots equally confirm that the selected model was adequate in predicting the response variables in the experimental values.

Characteristics of scent leaf oil

The fatty acid composition of scent leaf oil was analysed by gas chromatography mass-spectrometry (GC– MS). Table 3 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. Table 4 shows results for the physico-chemical properties of the extracted oil. 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 favourably compared with leaf oil of other plants such as olive leaf (49.86%) wt (Sudamalla et al., 2012; Ebewele et al., 2010). On the basis of the oil contents,

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

Runs	Independent variables				Responses
	A(l m)	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	1.00	0.00	0.00	0.00	31.67
4	1.00	0.00	2.00	0.00	43.56
5	1.00	-1.00	-1.0	1.00	20.14
6	1.00	0.00	0.00	0.00	31.62
7	1.00	-1.00	-1.00	-1.00	15.43
8	1.00	-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	1.00	0.00	-2.00	0.00	20.81
12	1.00	-1.00	1.00	-1.00	19.66
13	1.00	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

Table 4: Fatty acid composition of scent leaf 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}	Methylstearate	14.22
C_{25}	Pentacosylic acid	3.70
C_{30}	Melissic acid	5.75

O. gratissimum would be highly suitable for consumption.

A very negligible or no risk of fire outbreak when used as biodiesel or spills in the case of accident was obtained. A value of 1.441 was obtained for the refractive index. The refractive index value obtained falls within the range (1.447 to 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. (2010) reported that the specific gravity of scent leaf oil was found to be 0.892 at 25°C and this value is in the range found for other common oils. The SG value is also within the range (0.860 to 0.900) stipulated by EN14214 for bio-diesel (Ibeto et al., 2012). Iodine value of 31.09 ml eqg^{-1} was obtained.

GC-MS analysis of *O. gratissimum* leaf

Iodine value measures the degree of unsaturation of scent leaf oil. Oils with iodine value above 135 are classified as drying oil, while those with iodine value 110 to 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%) (Table 4), thus, affirming the oil largely consists of saturated fatty acids and is non-drying.

Peroxide value of 9 ml eqg^{-1} was obtained for the oil. Peroxide value was affected by several conditions which include oxidation by oxygen, extraction methods and storage. The low peroxide value suggests that scent leaf oil is stable to oxidative degradation caused by over exposure to oxygen, heating and improper storage (Silva et al., 2010; Basumatary et al., 2012). Saponification value of 32.258 ml eqg^{-1} was obtained for the sent leaf oil. The saponification value when compared with the values for common oils was very low: palm oil (196 to 205), groundnut oil (188 to 196), and corn oil (187 to 196) (Sangay et al., 2014). 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

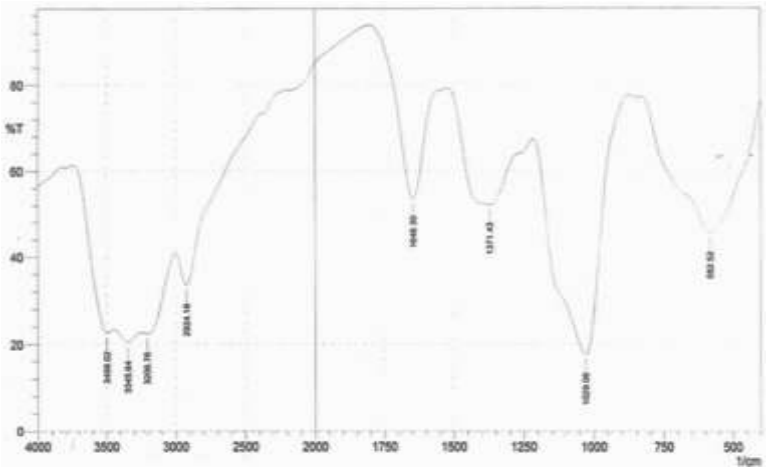

Figure 8: FTIR analysis of *O. gratissimum* oil.

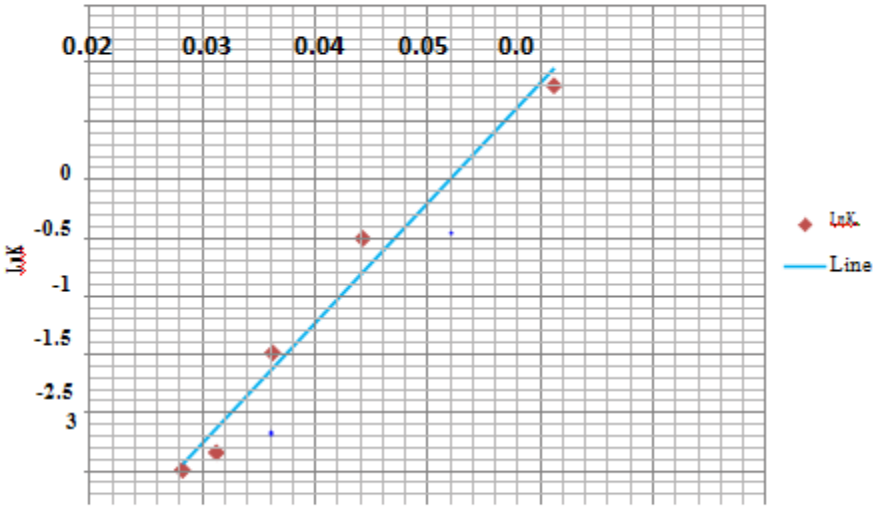

Figure 9: A plot of lnk versus I/T.

of the oil as compared to short chain fatty acids. Thus, the oil is not suitable for soap production. An acid value of 0.0336 mg KOH 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.

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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 1,646.3 cm^{-1}. The straight chain of– CH–stretch in aliphatic compound is found at the band 2,924.18 cm^{-1}, while the alkene group (CH=CH) is attributed to the band of 3,206.78 cm.

Kinetic study on extraction of scent leaves oil

Linearization of the Arrhenius law as shown here gives the values of the activation energy (E) and the temperature independent factor (A) from ln(k) against 1/T plot (Figure 9). The plot of ln(k) against 1/T gives ln(A) as the slope and $\frac{E}{R}$ as the intercept. A plot of ln(dY/dt) versus lnY was found to be linear. Figure 10 shows a second order kinetics

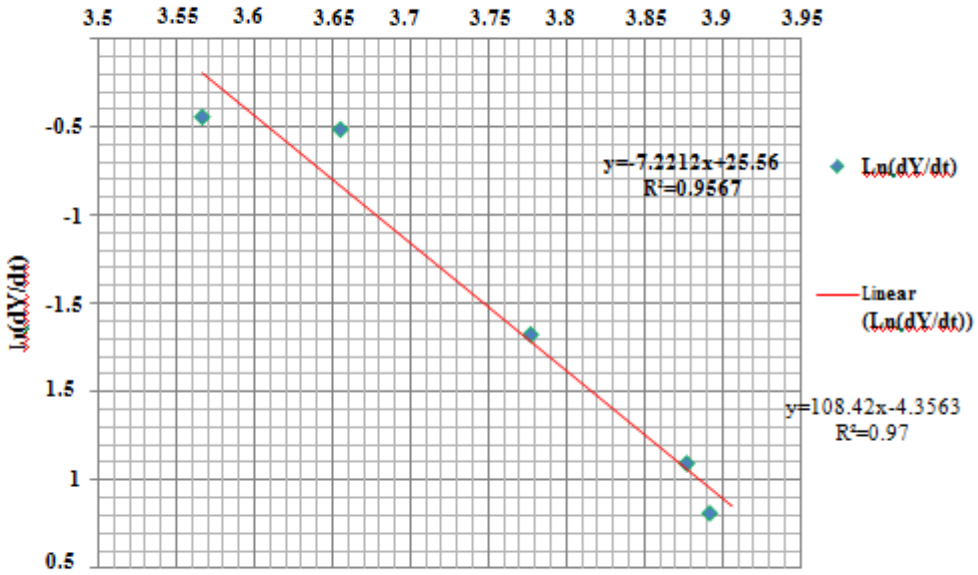

Figure 9: A plot of ln(dY/dt) versus lnY.

obtained from the slope of the straight line. The reaction rate constant was determined from the intercept as:

1.2610^{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.

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 fulfilment 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 accompanied 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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