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Composition and Radical Scavenging Activity of Essential Oil from *Xylopia aethiopica:* A New Chemotype Grown in Nigeria

Ignatius A. Owokotomo, Jamiu M. Jabar, and Omotayo O. Alabi

ABSTRACT

Despite its use in traditional medicines, studies on the radical scavenging activity of the essential oils from *Xylopia aethopica* from Nigeria have not been widely reported in literature. Thus, in this work, fresh fruits of *Xylopia aethopica* were air dried, ground and extracted through hydro-distillation using Clevenger-type distillation apparatus. The essential oil was analyzed using Gas Chromatographic/Flame Ionization Detection (GC/FID) Technique and confirmed by gas chromatographic/mass spectrometric (GC/MS) analysis. The radical scavenging activity of the essential oil was evaluated in comparison with butylated hydroxylanisole (BHA) through 1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging assay. A total of Forty-five compounds were detected through the GC/FID and GC/MS analyses of the essential oil of *Xylopia aethopica*. The major constituents of the essential oil were β-pinene (55.15%), α-thujene (9.23%) and α-eudesmol (8.61%) and α-pinene (6.77%). Other notable constituents were 1, 8-cineole (6.13%), ethyl cinnamate (5.83%) and elemol (5.17%). The methanolic solution of the essential oil showed concentration-dependent scavenging activity on 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) radical. The results of the Scavenging effect of the *Xylopia aethopica* essential oil compared well with the activity of the commercial radical scavenging agent, Butylated hydroxylanisole (BHA) and this suggests possible uses of the essential oil as sources of natural antioxidants. Data were analyzed using R statistical software.

Keywords: Essential oils, Radical scavenging activity, Xylopia aethiopica, Nigeria.

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I. Introduction

Essential oils (Floral scents) are volatile substances that carry distinctive odours or essence of plants. They can be referred to as active secondary metabolites from plants because they are very potent at trifling amounts which due to large number of compounds that often define their composition [1], [2]. Floral scents are presumed to have evolved during the course of evolution to guarantee subsistence, and this could explain their dual functions in many plants - they attract pollinators but also contain compounds that act to dissuade herbivory [3].

Nigeria is rich with many essential oil bearing plants among which is *Xylopia aethopica* commonly called Negro pepper and known locally as 'Erunje' in South-West Nigeria. It is a dense forest tree common to West Africa and often found growing in the wild especially along river banks and marshlands [4]. It belongs to the family of Annonaceae, sub-family Annonoidae, tribe is Unoneae, subtribe, Xylopinea [5].

The essential oil from *Xylopia aethopica* is commonly used against colds and as stimulants and stomachic [2]. Extracts from the plant are used as flavorings in fruit drinks and in traditional medicines in the treatment of arthritis, rheumatism, diarrhea, and dysentery [6]. It is also used in ethno medicine as a lactation stimulant [7], and in the treatment of female sterility and abdominal pains [8]. However, there is the concern that *Xylopia aethopica* may compromise male fertility [9], [10].

The chemical compositions of essential oils from Xylopia aethopica have been assigned by various authors. In a report, sabinene, 1, 8 – cineole, terpinen – 4 - ol and linalool were the major constituent of the essential oil of *Xylopia aethopica* from Benin, West Africa [11]. Another report confirmed the presence of β-pinene, γ-terpinene, trans-pinocaveol and p-cymene as the major constituent of a sample from Mali [12]. While α-pinene, β-pinene, sabinene, germacrene and 1, 8 – cineole was assigned as the major constituent of a Togo variety [13].

Earlier studies of the essential oils from some samples of *Xylopia aethopica* from Nigeria have been reported as consisting of α-pinene and β-pinene as the major chemical principles [14]. A different researcher identified the presence of 1,8-cineole and terpinen-4-ol as the major constituents of another sample of *Xylopia aethopica* from Nigeria [15]. Also, recently sabinene, eugenol, acetyl-eugenol, 1,8-cineole were identified as the major constituent of yet another sample from the country [16]. However, literatures on the radical scavenging activity of the essential oils from this plant from Nigeria are not common.

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Disequilibrium between the production of reactive oxygen species (O_2*, OH*, RO*, ROO* and H_2O_2) and the antioxidant capacity of natural antioxidants (superoxide dismutase, catalase, glutathione, peroxidase, vitamin C, vitamin E and β-carotene) in the human body leads to oxidative stress, which contributes to the genesis of a large number of pathological frames, resultant from structural damages caused by the oxidation of protein and lipid peroxidation [17].

Endogenous antioxidants like superoxide dismutase, catalase, glutathione peroxidase, uric acid, bilirubin, albumin, provide the natural defense against free radicals. However, exogenous antioxidants like vitamin E, vitamin C, β-carotene, vitamin E, flavonoids and synthetic compounds, like butylhydroxyanisole (BHA), butylhydroxytoluene (BHT), gallates, etc., may be required when a complete protection of the organism cannot be achieved by the endogenous factors [18], [30]. However, due to environmental concern and fret over the toxic side effects of synthetic antioxidants like butylated hydroxylanisole (BHA) and butylated hydroxyltoluene (BHT), several studies have in recent years, focused on the uses of essential oils as potential sources of safe antioxidants [19].

The free radical method using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) is a well-established assay for the in-vitro determination of antioxidant activity in food and biological extracts [31]. DPPH• (1,1-diphenyl-2-picryl-hydrazyl) is a stable free radical, due to the delocalization of the spare electron on the whole radical molecule. When DPPH• reacts with a hydrogen donor, the reduced (molecular) form (DPPH) is generated, accompanied by the disappearance of the violet colour, with an absorption band with a maximum around 520 nm [30].

Thus in this study, we investigate the chemical constituents and the radical scavenging activity of the essential oil from the fruits of *X. aethopica* grown in South West Nigeria.

II. Materials and Methods

A. Sample Collection & Preparation

The fruits of *X. aethiopica* were purchased from a farmer at Ado-Ekiti, Ekiti State, Nigeria (Fig. 1) and were air dried in the laboratory for three weeks. Fig. 1 shows the picture of the dried sample of *X. aethiopica* used in this experiment.

B. Essential Oil Extraction

The dried fruits of *X. aethiopica* were pulverized using a wooden mortar and pestle. The pulverized fruits were transferred into a two liter round bottom flask which was connected to an all-glass Clevenger-type distillation apparatus. The sample was distilled continuously for three hours until there was no appreciable increase in the volume of essential oil. The essential oil was collected into an airtight sample bottle and stored in the refrigerator without any further treatment prior to analysis.

Fig. 1. Dried sample of *Xylopia aethiopica* fruits.

C. Chemical analysis of the Essential Oil

Chemical analysis of the extracted essential oil was achieved through a Perkin-Elmer Auto System (HP 6890/ HP ChemStation Rev. A09.01 (1206) Software) GC, integrated with a dual Flame Ionization Detection (FID) system. The oven temperature was programmed from 40 ºC to 200 ºC for 2 minutes. The detector temperature was maintained at 300 ºC. The injection type was a split mode system and hydrogen was the carrier gas set at a flow rate of 1.0 mL/minute.

The GC/MS analysis was achieved through a Shimadzu GC-MS (QP5050A) series. The column capillary was DB-5; 30.0 m (length) × 0.25 mm (diameter) × 0.25 µm (film thickness). Helium was the carrier gas running at constant flow rate of 2.0 mL/min. Temperature was set 80 °C and held for 2.0 minutes and increased by 10 °C/min to a final temperature of 240 °C for 6.0 minutes. Sample injection volume was 1.0 µL. The spectrometric data were integrated through AMDIS (Automated Mass Spectral de-convolution and identification System) software. The retention indices (RI) were in relation to a homologous series of n-alkanes (C_7–C_{30}) on the DB-5 column under the same chromatographic conditions. Essential oil compositions were identified by comparison of the mass spectral fragmentation data with the NIST standard data bank (NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library, version 2002 to 2006) and data from literature [32].

D. DPPH Radical Scavenging Activity Analysis of the Essential Oil

The DPPH radical scavenging activity analysis of the essential oil of *X. aethiopica* was carried out using a procedure described in literature [33], with some modification [20]. The procedure involved preparing different concentrations of the essential oil in the range of 5.0 mg/mL, 10.0 mg/mL up to 30.0 mg/mL using methanol. A stock solution (50.0 µL) of each sample was then placed in a cuvette and 2.0 mL of 60.0 µM solution of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) solution in methanol was added and absorbance measured at 517 nm using a uv-visible spectrophotometer after 60 minutes of incubation. The same procedure was followed to determine the scavenging effect of butylated hydroxylanisole (BHA) on the DPPH radical to compare with that of the essential oil. A blank sample

containing only methanol and DPPH solution was also tested as a control. All readings were taken in triplicate and the percentage scavenging effect on the DPPH radical was then calculated as follows:

$$\%\ DPPH\ Scavenging\ effect = \frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{Absorbance\ of\ Control} \times 100$$

E. Statistical Analysis

We performed Analysis of variance (ANOVA – [14]) to check if there is a significant difference in the average effect of the two treatments (BHA and Essential oil of *X. aethiopica*) on 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH·). Although we have only two treatment groups, we decided on ANOVA because of the presence of a dose effect with more than two groups. Moreover, the ANOVA technique allows estimating the possible interaction between the treatment groups and Dose. In other words, we can examine if the average effect of the two treatments differs by dose groups with ANOVA. Furthermore, we performed a post-hoc analysis using the results from the ANOVA to establish the superiority of a treatment [14]. Finally, we tested to ensure that all assumptions of ANOVA were satisfied (see appendix A for the ANOVA model formulation and assumptions). All analysis was performed using R statistical software at 5% level of significance.

III. Results and Discussions

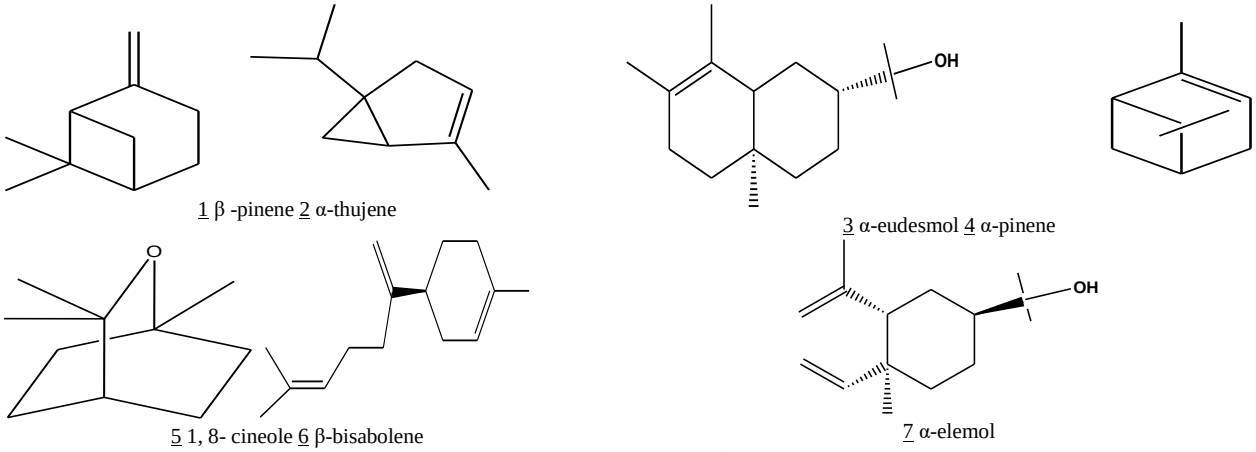
A. Chemical composition of the Essential oil

The Gas Chromatography-Flame Ionization Detection (GC/FID) and GC/MS analyses revealed the presence of forty-five chemical compounds (Table I). Only seven (Fig. 2) were present in appreciable quantities with the remaining thirty-eight occurring below 0.01%. The major constituents of the essential oil identified were β-pinene (55.15%) **1**, α-thujene (9.23%) **2**, and α-eudesmol (8.61%) **3**, and α-pinene (6.77%) **4**. Others were 1, 8-cineole (6.13%) **5**, β-bisabolene (5.83%) **6** and elemol (5.17%) **7**. The following are the chemical structures of the major compounds present in the essential oil of *X. aethiopica*.

The presence of α-pinene, β-pinene and 1,8-cineole as the major constituents of the essential oil from the present sample of *X. aethiopica* denotes a similarity with the previous reports from Nigeria [14]-[16]. However, the presence of thujene, elemol, β-bisabolene and eudesmol in significant amounts presents a major alteration. Thus, it may be inferred that the current sample of *X. aethiopica* is a different and new *chemotype* that is now reported for the first time.

TABLE I: Chemical Constituents of the Essential Oil of the Fruits of *Xylopia aethiopica*

S/N	Chemical compounds	Retention time	Retention index Percentage	(Rt) (RI) composition (%)
1	O-cymene	6.35	1041.0	0.0013
2	sabinene	7.29	973.0	0.0013
3	α-Pinene	9.69	936.1	6.77
4	β-Pinene	11.37	977.7	55.15
5	2-carene	12.09	1001	0.00071
6	myrecene	12.31	994	0.0011
7	E-ocimene	12.52	1050	0.0032
8	δ-limonene	12.83	1039	0.0012
9	allo-ocimene	13.51	1117.8	0.0016
10	(1S)-1(-1)-pinene	13.82	981	0.0013
11	Z-ocimene	14.06	1040	0.00024
12	α-thujene	14.50	931	9.30
13	elemol	15.02	1547.5	5.17
14	geranial	15.39	1270.3	0.0016
15	γ-terpenene	15.98	1059.7	0.0034
16	iso-artemisia	16.67	1083.1	0.0013
17	geraniol	17.19	1270.3	0.0013
18	nerol	17.50	1228.9	0.0018
19	eugenol	17.53	1335	0.0013
20	1,8-cineole	17.70	991	6.13
21	linalool	18.01	1098	0.00056
22	α -terpineol	18.68	939	0.0018
23	terpenen-4-ol	18.77	1177	0.0015
24	thymol methylether	19.73	1235	0.0017
25	α-farnesene	20.42	1508	0.0017
26	thymyl ethylether	20.79	763	0.0024
27	β-farnesene	21.40	1458	0.0031
28	linalyl acetate	21.52	1327	0.0039
29	ethyl cinnamate	21.99	1460	5.83
30	β-sesquiphellandrene	22.22	1149	0.0034
31	β-bisabolene	22.57	1509	0.002
32	(E,E)-fernesol	22.75	1721	0.0028
33	β-caryophyllene	22.90	1418	0.0026
34	spathulenol	23.35	1575	0.00076
35	t-α-bergamotene	24.02	1434	0.0030
36	β-elemene	24.44	1375	0.0030
37	bicyclogermacrene	25.26	1494	0.0025
38	α-copane	25.75	1376	0.0011
39	acetyleugenol	26.95	1520	0.0011
40	elemicin	27.07	1554	0.053
41	α-humulene	27.48	1455	0.00090
42	gamma muurolene	28.02	1477	0.0021
43	α-selinene	28.76	1494	0.0055
44	α-eudesmol	29.22	1652	8.61
45	velerinol	29.91	-	2.91
	Total			100.0%



1 β -pinene 2 α-thujene

3 α-eudesmol 4 α-pinene

5 1, 8- cineole 6 β-bisabolene

7 α-elemol

Fig. 2. The major constituents of *X. aethiopica*.

B. DPPH Radical Scavenging Activity of the Essential Oil

To obtain an overview of the average effect of the two treatments, and dose on DPPH radical, we obtained the average DPPH radical for the two treatments and also for each dose. We present these in Fig. 3. The average of DPPH radical for BHA is higher than that of the essential oil, while the variability of the two treatments is quite similar (panel A in Fig. 3). Although, this is without taking the dose effect into account, this already points at a potential difference in the effect of both treatments on DPPH radical. Regarding the dose effect on DPPH radical, the average DPPH radical scavenged increased with increasing dose. However, the average DPPH radical scavenged as well as the variability around those means for the last three dose levels (20, 25, 30 mg/ml) are essentially equal (panel B in Fig. 3). The variability in DPPH radical is highest at 10 mg/ml and lowest at 5 mg/ml. Panel C in Fig. 3 shows the interaction plot. Generally, BHA has a higher average DPPH radical scavenged compared to the essential oil irrespective of the dose. This implies there might be no interacting effect of the dose and treatment on the scavenging effect.

The test of the interaction effect was done in order to confirm if indeed there is no interaction between the dose and the effect of the treatments on the DPPH radical. To achieve this, we compared two ANOVA models, one with the interaction effect (model 2) and another without the interaction effect (model 1). The p-value of the test is 0.1642, which is greater than our specified level of significance. Thus, we do not have sufficient evidence to reject the null hypothesis so we can conclude that there is no interaction effect of the treatment and dose on DPPH radical (Table IV in appendix). We proceed to use model 1 for the rest of the analysis. The treatment and the dose have a significant effect on the effect on DPPH since the test for the difference in means for both variables have p-values lesser than the specified level of significance (Table II). This implies that the mean DPPH scavenged by the BHA is significantly different from the essential oil. Also, the mean DPPH scavenged is different per dose.

TABLE II: REDUCED ANOVA. TREATMENT AND DOSE EFFECT

	Mean SQ	F-value	P-value
Treatment	1.62255	247.51	0.00
Dose	0.73948	112.80	0.00
Residuals	0.00656		

Mean Sq = Mean squares. The null hypothesis is no significant difference in the means for the treatments and dose.

All ANOVA assumptions such as normality of the error term and constancy of variance were satisfied for (See appendix C).

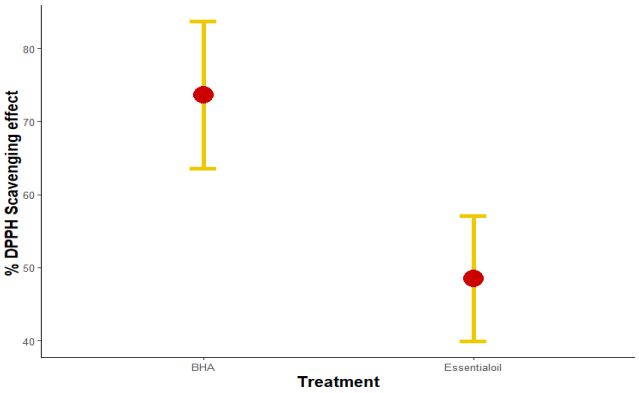
TABLE III: COMPARISON OF TREATMENT MEANS

	Estimated means	STD. error	T-value	P-value
BHA	3.99			
Essential oil	3.57			
Difference	0.42	0.03	15.73	0.00

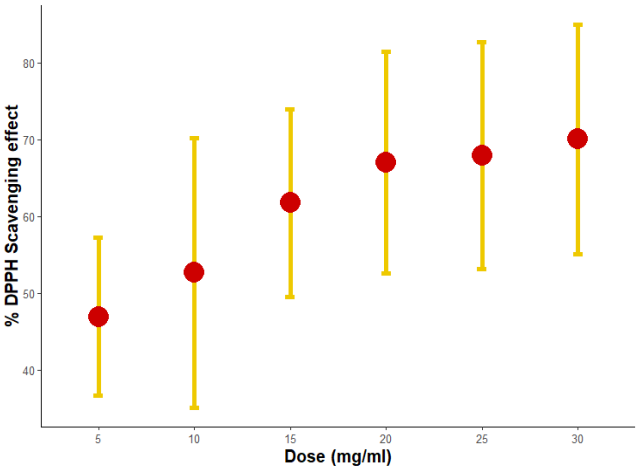
The null hypothesis is testing that the mean DPPH scavenged for BHA is similar to that of essential oil against an alternative that the mean of DPPH scavenged for BHA is significantly higher than that of essential oil.

The post hoc analysis presented in Table III shows that essential oil has a significant lower effect as compared to the BHA on the DPPH radical since the p-value for the null hypothesis indicates that there is sufficient evidence that the mean of BHA is significantly higher than that of essential oil.

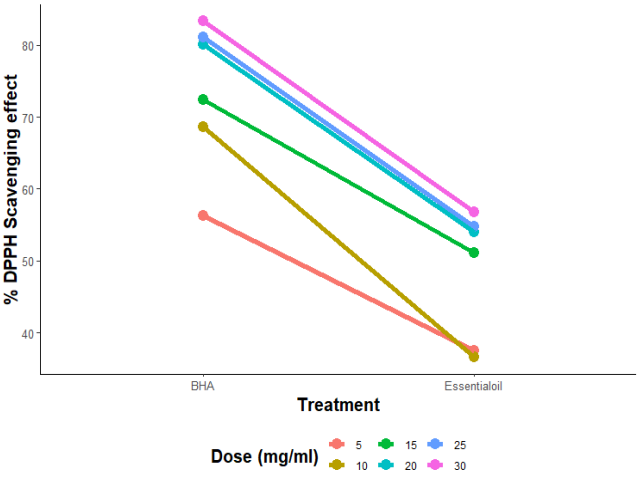
However, the scavenging effect of *X. aethiopica* essential oil fared comparably with the commercial radical scavenging agent, Butylated hydroxylanisole (BHA). The synergistic functions of the various components of essential oils may be the reason for their potency [21]-[23]. Thus, the presence of a vast number of compounds with diverse functionality in this essential oil may be the reason for its potent effect on the DPPH radical.



Panel A



Panel B



Panel C

Fig. 3. Average Radical per treatment (A), per dose (B), and per treatment-dose combination. (Each point is the computed mean, while the vertical lines in A and B are the variability around the computed means).

IV. Conclusions

The results obtained from this research indicate that the essential oil composition of the current sample of *Xylopia aethiopica* differs from previous reports in literature and may be classified as a new chemotype. The fruits of *Xylopia aethiopica* possess natural radical scavenging agents. The anti-radical potency of the essential oil was comparable to that of the synthetic antioxidant, BHA. The antioxidant activity of the essential oil may be as a result of the synergistic effect of large number of compounds (45 compounds) present in the essential oil. More so, essential oils are composed of terpenes and phenolic compounds which are known to have antioxidant properties [24], [25]. This research showed that *Xylopia aethiopica* fruit essential oil is a potential source of phytochemicals with useful pharmaceutical applications.

Appendix

A. ANOVA Model Formulation

The % Scavenging effect on DPPH radical was assumed to follow a normal distribution as this is a continuous outcome. Let Y_ijk be the % DPPH Scavenging effect for the kth observation (k=1,2,3) for the i^{th} treatment (i= 1,2) at the j^{th} dose level (j=1,2,3,4,5,6), then using the cell means formulation of the analysis of variance

Y_ijk = \mu_ij + \epsilon_ijk…….model 1
\mu_ij = \mu… + \alpha_i + \beta_j + (\alpha\beta)ij

where \alpha_i is the the effect of the i^{th} treatment effect, \epsilon_ijk are independent N(0, \sigma2)
i=1,…,a; j = 1,…,b; k=1,…,n

The model formulation in (1) assume that:

1) The parameter \mu_ij is the mean % Scavenging effect on DPPH radical at tratement j and dose level i, this is because E(\epsilon_ijk) = 0 and therefore E(Y_ijk) = 0.

2) Since \mu_ij is a constant, the variance of Y_ijk is:

\sigma2(Y_ijk) = \sigma2(\epsilon_ijk) = \sigma2 .

3) Since the error term are normally and independently distributed, so are the observations Y_ijk. Hence, the ANOVA model in equation (1) can also be stated as follows:

Y_ijk are independent N(\mu_ij, \sigma2).

B. Extra Tables

1. Test of interaction effect

H_0: There is no difference between models 1 and 2. Hence, there is no significant interacting effect of the treatment and dose on DPPH.

TABLE IV: Model Comparison between the Model without Interaction (Model 1) and that with the Interaction Effect (Model 2)

Model	DF	RSS	F	Pr(>F)
1	32	0.20344	2.027	0.1642
2	33	0.21633		

C. Test for ANOVA Model Assumptions

H_0: The variance of the residuals from the model is constant.

TABLE V: Test for Constancy of Variance for Model 1

Breusch Pagan Test for Constancy of Variance	
DF	1
X^2	1.74
P-VALUE	0.19

H_0: The error term from model is normally distributed.

TABLE VI: Test for Normality of the Residuals for Model 1

Test for Normality Assumption		
Test	Statistic	P-value
Shapiro-Wilk	0.95	0.15
Kolmogorov-Smirnov	0.12	0.67
Anderson-Darling	0.47	0.24

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