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Original Research Article

Comparison of Three Different Extraction Methods for the Determination of the Quality of the Leaf Volatile Oils of *Delonix regia* (Bojer ex Hook.Raf).

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ABSTRACT

Essential oils from natural sources form a new range of potent antimicrobial and antioxidant agents, hence, there is the need to explore the best method for their extraction, which retains the bioactive components of these oils. This research studied the effect of three different extraction methods on the yield, chemical composition, hence, quality of the volatile oils from the leaves of *Delonix regia*. Three different methods (hydrodistillation, steam distillation and cold maceration) were employed in the extraction of the oils from the leaves of *Delonix regia* plant. The extracted oils were subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The yield of oils obtained from the hydrodistilled, steam distilled and macerated air dried leaves were 0.32%, 0.54% and 1.25%, respectively. A total of 22, 22 and 12 compounds were identified using Gas chromatography in the hydrodistilled, steam distilled and macerated air-dried plant materials, respectively. Maceration of plant in n-hexane gave the highest oil yield (1.25%) compared to hydrodistillation (0.32%) and steam distillation (0.54%). The volatile oil of *Delonix regia* was composed mainly of monoterpenoids and sesquiterpenoids. The various extraction methods gave oils with varying major constituents- hydrodistillation, thymol (40.09%), steam distillation, caryophyllene (41.64%) and Cold maceration, Benzene (1,1-dimethylethoxy) (61.90%). The result revealed that the different extraction methods affected the yield, chemical composition and hence, the quality of the volatile oils of *Delonix regia*.

***Keywords*:** *Delonix regia*, Extraction, Caryophyllene, Thymol, Volatile oil.

Introduction

Delonix regia (Bojer ex Hook.) Raf., also known as Royal *Poinciana*, Flamboyant and Flame tree is a species of flowering plant, belonging to the family *Caesalpiniaceae*.[1] Different parts of the tree are used in traditional medicine.[2] It has been used in the folk medicine for the treatment of constipation, inflammation, arthritis, hemiplagia, leucorrhoea and rheumatism.[3,4] The bark and flowers of *D. regia* have been reported to have broad spectrum antimicrobial activity and anti-inflammatory properties.[5,6] The leaves and flowers of *D. regia* showed strong phytotoxicity against *Mikania micrantha*.[7] Allelopathic potential of leguminous plant species towards *Parthenium hysterophorus* was tested by using aqueous foliar leachates, and was strongest with leachates from *D. regia*.[8,9] The chemical composition of the polar and non-polar extracts of *Delonix regia* have been reported. Volatile compounds such as 3-carene, (R)-(+)-m-mentha-6,8-diene, (1R)-(+)-α-pinene, octadiene, and polar compounds such as stigmasterol, phytol, sitosterol, ergost-4-en-3-one and ergost-5-en-3-ol, tetradecanoic acid, palmitic acid, erucic acid have been identified to be present in the essential oil and crude extracts of *D. regia*.[10,11]

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D. regia wood ash induced up to 78, 81 and 89% reduction in the mycelial growth of *Helminthosporium sativum, Curvularia lunata* and *Fusarium graminearum* respectively.[12] There is very little research report on the volatile components of the volatile oil of *D. regia* and no previous report on the effect of extraction methods. It is on this premise that this research seeks to study the effect of three different extraction methods (hydrodistillation, steam distillation and cold maceration) on the yield, chemical composition and quality of *D. regia* volatile oils.

Materials and Methods

Plant materials and volatile oil extraction technique

The healthy leaves of *Delonix regia* were collected from the botanical garden of the University of Lagos, Akoka,Yaba, Lagos State, Nigeria in August, 2019. The botanical identification and authentication was done at the Herbarium of the Department of Botany, University of Lagos, Nigeria with a voucher number LUH 6411 The fresh leaves were air-dried for a period of one week and pulverized using a mechanical grinder, MicronGlacis GC fine grinding mill prior to extraction. A batch of the oils from the pulverized leaves were obtained by the hydrodistillation of 300 g of the plant material using the modified Clevenger-type apparatus.[13] The second batch of pulverized leaves (300 g) was extracted using a steam distillation set-up for 3 hours, while the third batch of pulverized leaves was macerated in n-hexane for 72 hours and filtered. The oils obtained from the three batches were dried over anhydrous sodium sulphate Sigma-Aldrich,USA) and stored in a refrigerator prior to analysis.

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GC–FID and GC-MS analyses of volatile oils

The essential oil samples were analysed using a Varian CP-3800 gas chromatograph fitted with a flame ionization detector (FID) and dimethylpolysiloxane (100%) column (CP Sil-5 CB: 50 m length × 0.25 mm i.d. × 0.4 µm film thickness) (Varian, Netherlands). Nitrogen was the carrier gas with a 16-psi inlet pressure. Samples (0.2 µL) were injected in split mode with a ratio of 1:100. The column was initially held at 60°C for 5 minutes then heated to 220°C at a 5°C/minute ramp rate and was held for 3 minutes at that temperature. The temperature was further raised to 250°C at a 5°C/minute ramp rate and was held at this temperature for 4 minutes. The injector and detector temperatures were maintained at 250 and 300°C respectively. The gas chromatography-mass spectrometry (GC-MS) analyses performed on a Perkin Elmer Turbo mass Clarus 600 Instrument at 70 eV ionization energy with a mass range of 40–500 amu, employing an Elite-5 column (5% phenyl and 95% dimethylpolysiloxane) of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness (PerkinElmer, USA). Helium (1 mL/min) was used as a carrier gas. The initial temperature was 60°C (1 min), this was increased to 240°C at rate of 6°C/min, and remained at 240°C for 6 min, and then continued to increase to 250°C at rate of 10°C/min, with a final stage of 10 min at 250°C. The oven temperature was programmed from 50 to 250°C at a 5°C/min dynamic rate, and remained for 15 min at 250 °C. Samples (0.1 uL) were injected with a splitless mode.

Identification of volatile oil constituents

Component identification was accomplished by comparison of the retention indices of the GC peaks with those obtained using saturated n-alkanes (C8–C20) (Aldrich, USA), those reported in the literature[14-16] and by comparison of the mass spectra of the peaks with those reported in literature[17,18] and stored in the NIST library. Peak area percentages were calculated from GC–FID response without employing correction factors. Average values of three replicates along with their standard deviation values are presented and discussed.

Results and Discussion

The volatile oils of *Delonix regia* was extracted by three different methods- hydrodistillation, steam distillation and cold maceration. The leaf volatile oils of *Delonix regia* yielded 0.32, 0.54 and 1.25% on dry weight basis for hydrodistillation, steam distillation and cold maceration respectively.

The volatile oils were composed of majorly mono and sesquiterpenoids. A total of 56 constituents were identified and are listed in Table 1. The hydrodistilled oil had a total of 22 constituents (99.85 %), while the steam distilled and macerated oils had 22 (99.85 %) and 12 (99.93 %) constituents respectively (figures 1-3). The major constituents of the hydrodistilled oil were thymol (40.09 %), cis-muurola-4 (15),5-diene (4.93 %), δ-cadinene (4.04 %), and α-copaene (3.61 %). The major constituents of the steam distilled oil were caryophyllene (41.64 %), thymol (20.42 %), β-selinene (8.02 %), Isocaryophyllene (7.86 %), γ-gurjunene (3.45 %) and δ-cadinene (3.44 %), while the cold macerated oil was composed of benzene (1,1-dimethoxylethoxy) (61.90 %), epoxy linalool oxide (17.10 %) β-lonone (8.23 %) and 2,7-octadiene-1,6-diol-2,6-dimethyl- (6.21 %) as the major constituents. Five compounds were present in the hydrodistilled and steam distilled oils but absent in the macerated oils, however compounds showed noticeable variations in the distilled oils, they were: Thymol, α-copaene, caryophyllene, δ-cadinene and β-selinene. However, only one compound (benzene (1,1-dimethoxylethoxy) was present in macerated oil, but absent in the distilled oils. Changes in the nature and chemical composition of volatile oils have been attributed to methods of extraction. It has largely been reported that the volatile oil yield of medicinal plants varied with extraction methods. This is largely due to the fact that the characteristics of the components are redefined during the extraction process due to the controlled distillation in hydrodistillation and continuous cohobation of the distilled water.[19-21] The Chemical Composition of the polar and non-polar extracts of *Delonix regia* have been reported. Volatile compounds such as 3-carene, mentha-6,8-diene, (+)-α-pinene, octadiene, and polar compounds such as stigmasterol, phytol, sitosterol,ergost-4-en-3-one and ergost-5-en-3-ol, tetradecanoic acid, palmitic acid, erucic acid have beenidentified to be present in the volatile oil and crude extracts of *D. Regia* from Jamaica and Egypt.[11,22] These however varied in composition from the volatiles studied in this research.The oils also varied in the composition of grouped compounds. The distilled oils had higher percentages of sesquiterpenoids hydrocarbon while the macerated oil had higher percentage of oxygenated monoterpenoids. A lot of factors including water quality, pH and temperature might have caused hydrolysis, isomerization, racemization and oxidative altering of the structures of the volatile oil components, extracted by different methods.[23] The higher percentages of sesquiterpene hydrocarbon in distillation methods is noteworthy.

Table 1: The chemical composition of volatile oils of *Delonix regia* obtained by different extraction methods

Name	RI	RIcal	Hydrodistillation (%)	Steam distillation (%)	Cold maceration (%)	Standard Deviation
β-Myrcene	983	982	-	-	0.33	-
cis-p-Menth-8-ene	993	991	-	-	1.75	-
p-Cymene	1025	1026	-	3.59	-	-
α-Ocimene	1039	1040	-	0.10	-	-
5-Octen-2-one, 3,6 dimethyl-	1072	1073	0.53	-	-	-
Benzene, (1,1-dimethylethoxy)-	1083	1081	-	-	61.90	43.68
2-Isopropenyl-5-methylhex-4-enal	1092	1094	-	-	0.80	-
Terpinen-4-ol	1164	1161	0.65	-	-	-
trans-Piperitol	1192	1190	0.76	-	-	-
β-Cyclocitral	1197	1195	1.01	-	-	-
Thymol methyl ether	1214	1213	1.13	-	-	-
α-Ylangene	1221	1219	-	0.19	-	-
Epoxy-linalooloxide	1224	1222	-	-	17.10	-
Thymol	1270	1269	40.09	20.42	-	13.90
Bicyclo[5.2.1]decan-10-one	1273	1272	-	-	0.25	-

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Carvacrol	1279	1276	-	0.08	-	-
2,7-octadiene-1,6-diol-2,6-dimethyl	1367		-	-	6.21	-
α-Copaene	1376	1374	3.61	2.86	-	0.53
(-)-β-Bourbonene	1382	1382	-	0.17	-	-
β-Cubebene	1385	1385	-	0.03	-	-
Cedrene	1406	1411	-	0.99	-	-
Isocaryophyllene	1409	1408	-	7.86	-	-
Caryophyllene	1419	1416	31.54	41.64	-	7.14
β-Cedrene	1422	1420	2.02	-	-	-
cis-Geranylacetone	1429	1431	0.79	-	-	-
Isogermacrene D	1437	1431	-	0.09	-	-
α-Muurolene	1440	1439	1.92	-	-	-
cis-β-Farnesene	1445	1446	-	-	0.60	-
Selina-5,11-diene	1447	1447	2.47	-	-	-
cis-Muurola-3,5-diene	1448	1449	0.25	-	-	-
Humulene	1451	1451	-	0.39	-	-
cis-Muurola-4(15),5-diene	1454	1452	4.93	-	-	-
α-Patchoulene	1456	1457	-	0.04	-	-
δ-Cadinene	1469	1468	4.04	3.44	-	0.42
γ-Gurjunene	1470	1469	-	3.45	-	-
Germacrene D	1477	1471	0.13	1.61	-	1.04
β-Selinene	1482	1482	0.21	8.02	-	5.52
2,6,10,10-Tetramethylbicyclo [7.2.0]undeca-2,6-diene	1499	1493	-	3.12	-	-
γ-Cadinene	1507	1506	0.61	-	-	-
cis-Z-α-Bisabolene epoxide	1531	1531	-	-	0.18	-
4-isopropyl-1,6-dimethyl-1,2,3,4-tetrahydronaphthalene	1537	1537	1.14	-	-	-
6-epi-shyobunol	1555	1554	0.25	-	-	-
β-Ionone, methyl-	1557	1551	-	-	8.23	-
Viridiflorol	1582	1582	-	-	2.25	-
Geranyl isovalerate	1585	1583	-	-	0.33	-
Ledol	1586	1587	-	1.09	-	-
Isoaromadendrene epoxide	1590	1589	2.69	-	-	-
β-copaene	1598	1599	0.08	-	-	-
E,E-6,8-Tridecadien-2-ol, acetate	1631	1631	-	0.56	-	-
(Z,E)-Farnesol	1681	1680	-	0.05	-	-
% Yield			0.32	0.54	1.25	-
Grouped Components						
Monoterpene hydrocarbons			-	8.70	18.18	
Oxygenated monoterpenes			22.73	13.04	36.36	
Sesquiterpene hydrocarbons			59.09	65.22	9.09	
Oxygenated sesquiterpenes			9.09	13.04	27.27	
Non terpenic components			9.09	-	9.09	
Total % of compounds identified			99.85	99.90	99.93	
Total number of compounds identified			22	23	11	

Notes: RIcal, retention index calculated on CP Sil-5 column relative to C8–C20 n-alkanes; RI, retention index reported in the literature.

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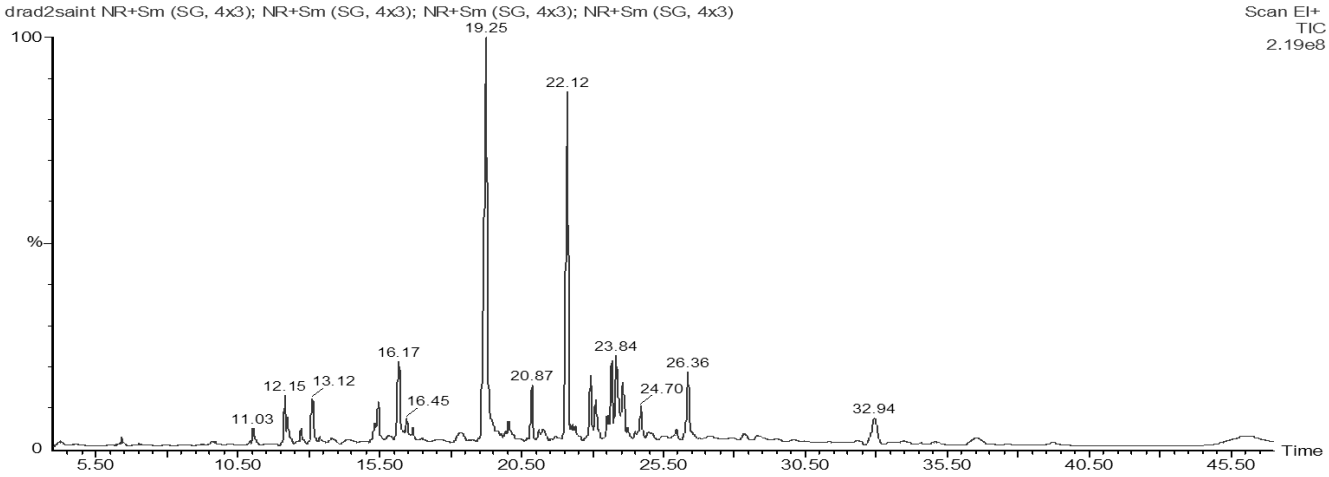

Figure 1: Total Ion Chromatogram of hydrodistilled oil of *D. regia.*

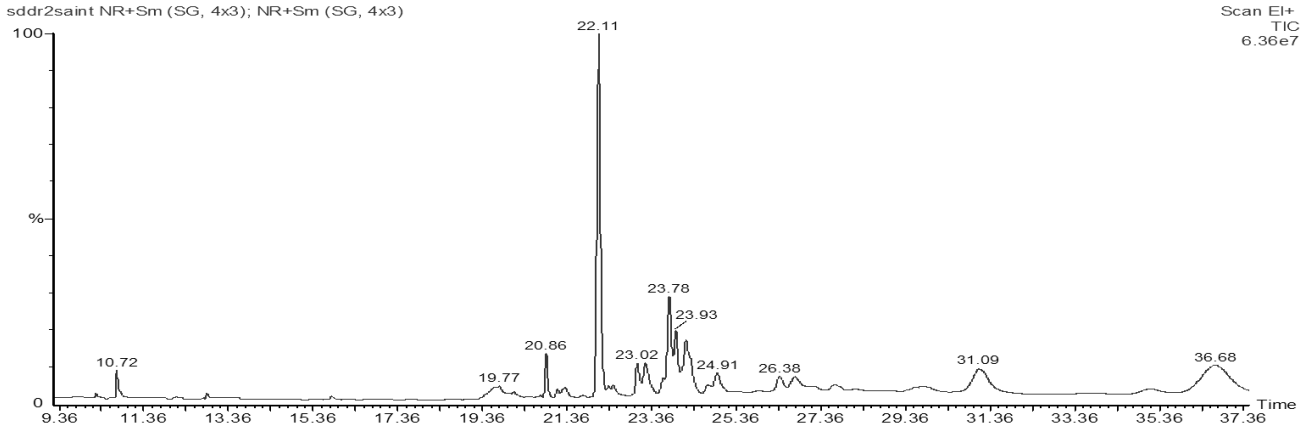

Figure 2: Total Ion Chromatogram of steam distilled oil of *D. regia.*

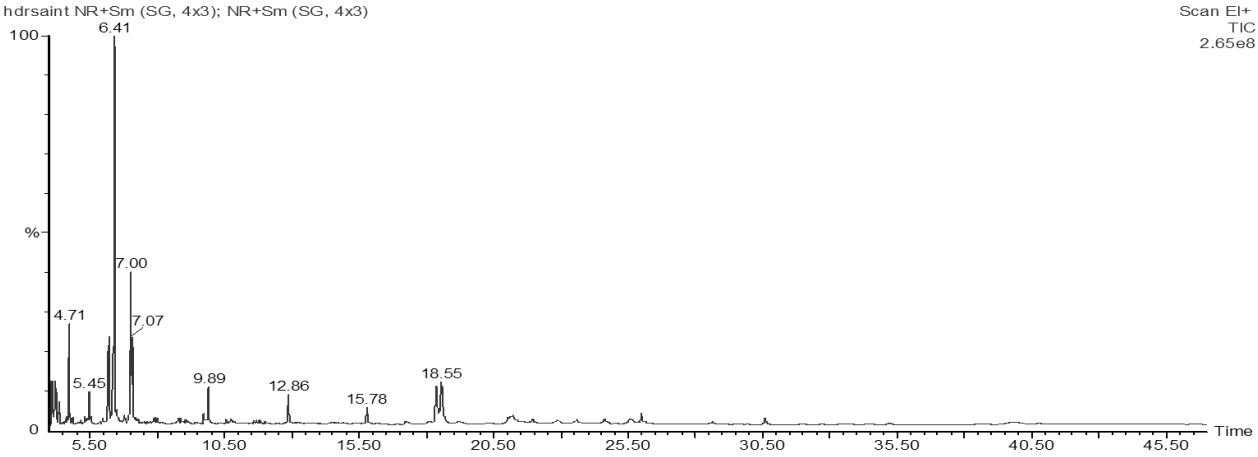

Figure 3: Total Ion Chromatogram of cold macerated oil of *D. regia.*

The most abundant components in the distilled oils were thymol (HD), caryophyllene (SD) and benzene, (1,8)-dimethylethoxy (CD). Distillation methods removed benzene, (1,8)-dimethylethoxy, a poisonous substance in the cold macerated leaf oil, as it was absent in the distilled oils. Also worthy of note is the presence in high percentage composition of caryophyllene and thymol in the steam and hydrodistilled oils, which increases the quality of those oils because of the biological importance of these compounds. The method of volatile oil extraction affects the quality and hence, the biological activities of the volatile oils of *D. regia.*[24] Earlier reports of thymol rich volatile oils and their biological activities are well documented.[25,26]The broad spectrum antimicrobial activity exhibited by thymol accounts for its use as general purpose disinfectant.[27]Caryophyllene has been reported to have anti-inflammatory, analgesic, anti-cancer, anti-anxiety and anti-depressant properties.[28,29]

Conclusion

The yield, chemical composition and quality of *D. regia* essential oil was affected by different extraction methods. Hydrodistillation and steam distillation remain the best of the three methods in this study.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

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