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Chemical constituents and anti-inflammatory activity of essential oils of *Datura stramonium* L.

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Abstract
The chemical constituents and anti-inflammatory activity of essential oils of *Datura stramonium* L. (family Solanaceae) are being reported. The essential oils were obtained by separate hydrodistillation of the air-dried and pulverized leaves and seeds of *D. stramonium*. The essential oils were analysed by means of gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). (72.5%) was identified as the main constituent of the leaf oil. However, citral (26.5%), 4,8-dimethyl-3,8-dien-2-one (11.2%), sesquirosefuran (11.1%) and geraniol (10.5%) were identified as the major constituents in the seed oil. The anti-inflammatory action was determined on fresh egg albumins over 4 h by measurement of baseline paw diameters. Oral administration of essential oils at a dose of 2% showed significant (p<0.001) anti-inflammatory properties in the albumin-induced test model in rats compared to the control, while activity as compared to the standard drug, Diclofenac (100 mg/kg), was only significant P<0.05 for the duration of the analysis. Oils from the leaves inhibited inflammation beyond 4 h post treatment with percentage inhibition ranging between 22 to 95% throughout the period of analysis. The potent anti-inflammatory activity of essential oils of *D. stramonium* may serves as exploitation of *D. stramonium* in treating various inflammatory diseases.

Keywords: *Datura stramonium*; essential oil; terpenes; anti-inflammatory activity

1. Introduction
Datura stramonium L. (Solanaceae) is an annual plant. The leaves are hairy with stalked 4-6 in long, ovate and pale green while the stem is herbaceous, branched, glabrous and lightly hairy. Fruits are as large as walnuts and full of thorns [1]. The whole plant is poisonous [2, 3]. In West Africa, the whole plants are used for anti-inflammatory and for treatment of dental pain and skin infections. The dried pulverized leaves are sprinkled on wounds or mixed with ointment for healing [4].

Extracts of the plant are known for their antiasthmatic [5], acaricidal [6], insect repellent [6], oviposition deterrent [6], antimicrobial [7, 8] and anticancer [9] activities. In addition, the anti-inflammatory [10, 11], analgesic, antidiarrhoeal [11], larvicidal [12], pesticidal toxicity [13], antifungal [14], vibriocidal [15] and anticonvulsant [16] potentials of the various extracts of the plant have been reported. The phytochemical compounds isolated from *D. stramonium* such as daturaolone, daturadiol, stigmasterol and sitosterol were shown to possess significant immunostimulatory activity [17]. *D. stramonium* contain variety of alkaloids including atropine, hyoscamine and scopolamine [18], tigloidin, aposcopolamine, apoatropine, hyoscyamine N-oxide and scopolamine-N-oxide [18, 19], 6α-ditigloyloxytropane and 7-hydroxyhyoscyamine[2], tropane esters 3-(3′-acetoxytropoyloxy)tropane and 3-(2′-hydroxytropoyloxy)tropane [19].

There are only few reports about the volatile components of the plant growing in China. The main components of the leaf oil [20] were identified as 5-α-ergosta-7, 22-dien-3-β-ol (16.53%), 3-hydroxycholestan-5-yl, acetate (14.97%) and 26, 26-dimethyl-5,24 (28)-ergostadien-3-β-ol (10.39%). In another investigation [21], the principal components of the oil were reported to be 6-pentyl-5, 6-dihydro-2H-pyran-2-one (44.29%), diphenylamine (12.50%) and tetratetracontane (10.41%). The fruit volatile oil [22] were shown to consist mainly of 6-pentyl-5, 6-dihydro-2H-pyran-2-one (9.13%), (*E*)- 3,7,11,15-tetramethyl-2-hexadecen-1-ol (6.71%), benzophenone (6.16%) and 1-hexanol (6.10%). Moreover, stramenlactone, (R)-tuberolactone, daturadiol, monolinoleoyl glycerol, linoleic acid and lutein were recently isolated from the essential oil of *D. stramonium* [23]. The essential oils of *D. stramonium* have been reported to display allelopathic [20], antibacterial [24], insecticidal [25] and antifungal [26] effects.

The aim of the present paper was to report the chemical constituents identified in the essential oils of the leaf and seed of *D. stramonium* and results of *in vivo* anti-inflammatory activity of the essential oils.

2. Materials and Methods

2.1 Materials

2.1.1 Chemicals

Unless otherwise stated, all chemicals and reagents were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). All the chemicals used were of analytical grade. Diclofenac injection was purchased from Lagos State University Pharmacy manufactured by May and Baker.

2.1.2 Animals

Wistar rats (8 weeks, 150 to 200 kg) of either sex were collected from the animal house in the Department of Biochemistry, Lagos State University, Nigeria. The animals were maintained under standard conditions (temperature 23 ± 2 ºC and 12h light dark cycle); had free access to standard pellet feed and enough drinking water. Ethic clearance certificate was obtained from the Research Ethical Clearance Committee (RECC) of the University (Approval no: 012/2016/LASU/BCH).

2.1.3 Plant materials

Fresh leaves and seeds of *D. stramonium* were collected from Odonla village, Ikorodu, Lagos State, Nigeria, in May 2016. The taxonomic identification of the plant material was confirmed by Curators at the Herbarium of the he Department of Botany, University of Lagos, Nigeria. A voucher specimen (LUH-7004) was deposited in the Herbarium for future reference.

2.2 Methods

2.2.1 Hydrodistillation of essential oils

Briefly, 223.0 g (leaves) and 2.2 g (seeds) of the pulverized sample were carefully introduced separately into a 5 L flask and distilled water was added until it covers the sample completely. Hydrodistillation was carried out separately in an all glass Clevenger-type distillation unit designed according to British Pharmacopoeia specification [27]. The volatile oils distilled over water and were collected in the receiver arm of the apparatus into a clean and previously weighed sample bottles. The oils were kept under refrigeration (4 ºC) until the moment of analyses.

2.2.2 Analysis of the essential oils

Gas chromatography (GC) analyses were carried out on a Hewlett Packard Gas Chromatography HP 6820 equipped with FID and HP-5MS column (60m x 0.25mm id), 0.25 µm film thickness and split ratio of 1:25. The oven temperature was programmed from 50 ºC (after 2 min) to 240 ºC at 5 ºC/min and the final temperature was held for 10 min. Injection and detector temperatures were 200 ºC and 240 ºC respectively. Hydrogen was the carrier gas at flow rate of 1 mL/min. 0.5 µL of the diluted oil was injected into the GC. Peaks were measured by electronic integration. *n*-Alkanes were run at the same condition for retention indices determination.

Gas chromatography-mass spectrometry (GC-MS) analyses were performed on a Hewlett Packard Gas Chromatography HP 6890 interfaced with Hewlett Packard 5973 mass spectrometer system equipped with a HP-5MS capillary column (30m x 0.25 mm id, film thickness 0.25 µm). The oven temperature was programmed from 70-240 ºC at the rate of 5 ºC/min. The ion source was set at 240 ºC and electron ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 1 mL/min. Scanning range was 35 to 425 amu. 1.0 µL of diluted oil in hexane was injected into the GC/MS. The identity of the oil components were assigned by comparison of their retention indices with the authentic samples and matching of their mass spectra with the Wiley 275 library mass spectra database as well as with published data [28].

2.3 Anti-inflammatory test

The egg albumin-induced hind paw oedema test was conducted with little modification according to established procedure [29]. Wistar rats were assigned to one of 3 groups consisting of 5 animals each as follows:

(i) group1-control (treated with water),
(ii) group 2- standard (treated with Diclofenac Sodium injection 100 mg/kg, orally),
(iii) group 3- treated with 2% essential oil suspension orally

The same treatment was repeated on day 2 and animals were starved for 8 h before experimentation. On day 3 of the experiment, same volume of vehicle was administered. Thirty minutes later, 1.0 mL of 50% (v/v) of fresh egg albumin was injected subcutaneously into the subplantar surface of the hind paw. Rat paw oedema was assessed by volume displacement method (plethysmometer Ugo Basile) before and after egg-albumin injection at 1, 2, 3, and 4 h. The changes in paw sizes were then evaluated.

From the mean edema volume, the percent inhibition was calculated by using following formula [30]

% Inhibition of edema = 100 x (Vc-Vt/Vc)

Where, Vc =Mean paw edema of control group

Vt =Mean paw edema of treated group

2.3.1 Statistical analysis

Repeated Measures One way ANOVA Analysis using Tukey's multiple comparisons Test was performed using GraphPad Prism (version 7.02), San Diego California USA, www.graphPad.com) to compare activity between treatment group, control and the standard. The *p* value was significant for $p>0.05$ and above values. Results were expressed as mean ± standard error of the mean.

3. Results

3.1 Chemical constituents of the essential oils

Hydrodistillation of the dried leaves and seeds of *D. stramonium* offered essential oils (EO's) in yields of 0.35% (w/w) and 0.01% (w/w) respectively, calculated on a dry weight basis. The obtained oils have light yellow color and a aromatic odor. The compositions of the EO's were presented in Table 1, where all compounds are listed according to their elution from a HP-5MS column. The GC chromatogram shows the presence of twenty-four volatile compounds of which nineteen were identified in the seed oil of *D. stramonium*, accounting for 93.2% of the total compounds. On the other hand, six compounds representing 97.0% of the total oil contents were identified in the leaf oil. The main classes of compounds present in the leaf oil were diterpenes (74.8%) and oxygenated monoterpenes (10.4%). On the other hand, oxygenated monoterpenes (41.4%), oxygenated sesquiterpenes (28.0%) and aliphatic ketones (13.9%) were the main classes of compounds present in the seed oil. Phytol (72.5%) was identified as the main constituent of the leaf oil.

Other significant compounds include 6,10,14-trimethy-2-pentadecanone (9.5%), levomenthol (6.1%), (E)-β-ionone (4.3%), octadecamethylcyclononasiloxane (2.5%) and 13-*apo*-β-carotenone (2.3%). However, citral (26.5%), 4, 8-dimethyl-3, 8-dien-2-one (11.2%), sesquirosefuran (11.1%) and geraniol (10.5%) were identified as the major constituents in the seed oil. Previous studies have identified mostly sterols [20], aromatic compounds [21, 22] and fatty acids [23] as the main dominant volatile compounds of of *D. stramonium*. Our results, in which terpene compounds predominates are at variance with previous studies on *D. stramonium* essential oil from China. This may be attributed to the differences in ecological and climatic conditions between the Nigeria and China.

Table 1: Chemical constituents of essential oil of *D. stramonium*

Compounds [a]	RI [b]	RI [c]	Seed	Leaf
Tetramethylethylene	630	630	3.3	-
2-Ethyl-4-methyl-1H-pyrrole	982	984	1.9	-
Linalool	1100	1095	0.1	-
Levomenthol	1174	1172	-	6.1
4,8-Dimethyl-3,8-dien-2-one	1245	1240	11.2	-
Citral	1251	1249	26.5	-
Geraniol	1268	1267	10.5	-
2-Undecanone	1293	1293	1.6	-
Geranyl acetate	1385	1383	4.7	-
2-Tridecanone	1493	1494	1.1	-
(E)-β-Ionone	1498	1496	-	4.3
Sesquirosefuran	1560	1557	11.1	-
(*E*)-Nerolidol	1561	1563	4.2	-
Caryophyllene oxide	1577	1578	1.2	-
Selin-11-en-4α-ol	1654	1651	2.3	-
α-Bisabolol	1690	1685	3.9	-
Octadecamethylcyclononasiloxane	1689	1690	-	2.5
α-Farnesol	1695	1695	0.3	
6,10,14-Trimethy-2-pentadecanone	1848	1847	-	9.5
(2*E*,6*E*)-Farnesyl acetate	1857	1854	5.0	-
m-Camphorene	1962	1960	1.4	-
Geranyl linalool	2040	2034	1.4	-
Phytol	2119	2129	-	72.5
13-*apo*-β-Carotenone	2125	2130	-	2.3
Geranylgeraniol	2204	2201	1.8	-
Total			93.1	97.0
Monoterpene hydrocarbons			-	-
Oxygenated monoterpenes			41.4	10.4
Sesquiterpene hydrocarbons			-	-
Oxygenated sesquiterpenes			28.0	9.5
Diterpenes			4.6	74.8
Aliphatic ketones			13.9	-
Aromatic compounds			5.2	-

[a] Elution order on HP-5MS column; [b] Retention indices on HP-5MS column; [c] Literature retention indices; - Not identified

3.2 Anti-inflammatory activity of the essential oils

Figure 1 illustrated the anti-inflammation effects of essential oils of *D. stramonium* on fresh egg albumin-induced inflammation measured at 1, 2, 3, and 4 h. Within the hours, essential oil activity showed significant (P<0.01) anti-inflammatory as compared to the control. Percentage inhibition shows 22.2%, 29.7%, 53.8% and 92.3% for 1st to 4th hour respectively.

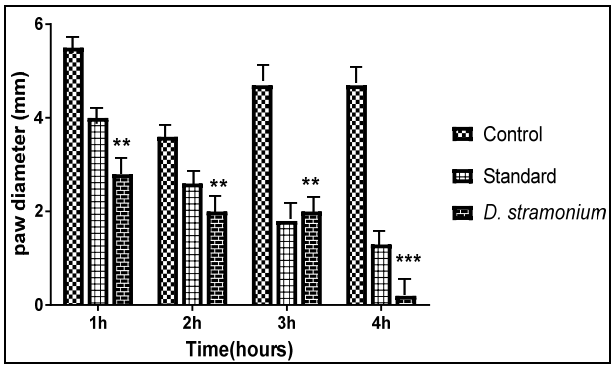

Fig 1: Anti-inflammatory activity of essential oil of *D. stramonium* on egg albumins over 4 h. The results are expressed as the mean ± S.E.M. of five rats. ***P*<0.01, **P*<0.05 compared to control animal

On the other hand, Fig. 2 showed a comparison of the anti-inflammatory effect of Diclofenac (standard) with the oils of *D. stramonium* oils showing a significant inhibition of (P<0.05) during the analysis period.

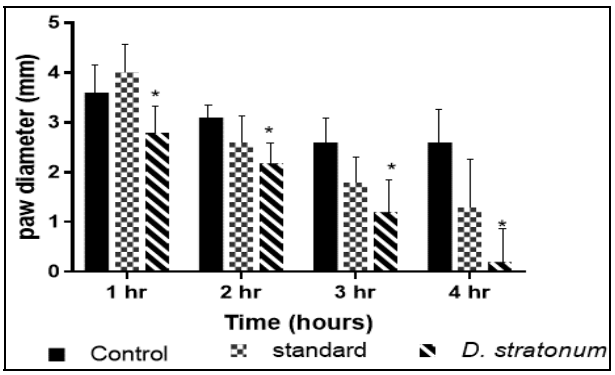

Fig 2: Anti-inflammatory activity of essential oil of *D. stramonium* on egg albumins over 4 h. The results are expressed as the mean ± S.E.M. of five rats. ***P*<0.01, **P*<0.05 extract compared to standard (Diclofenac injection 100 mg/kg) animal

Acute inflammation which occurs over a few periods of days, are influenced by release of some mediators in three (3) different time phases. Histamine and serotonin are released in the first phase during the first 1.5 h [31]. The second phase involves the release of bradykinin from 1.5 h to 2.5 h, whilst the last phase involves the release of prostaglandins between 2.5 h to 6.0 h phlogistic administration [32].

Oils from the leaves inhibited inflammation beyond 4 h post treatment while the seed oil was inactive. The potency of the leaf oil shows it has a very high potency to increase the mediation of histamines, serotonin, prostaglandins and bradykinins due to its high activity over the three (3) phases of acute inflammation. The biological activity of an essential oil has been attributed to the potency of the major compound or a synergy between both the major and minor compounds. Certain terpenes had been reported to be exhibit high anti-inflammation property. The presence of large amounts of phytol in the essential oil of the leaf might be the factor responsible for the anti-inflammatory activity. This terpene has strong anti-inflammatory effect by inhibiting the COX1 and the leukotriene [33, 34]. In addition, leaf ketones also exhibit this property which in this case could be attribute to the 6, 10, 14-trimethy-2-pentadecanone and (*E*)-β-ionone present in appreciable amount in the oil. The potent anti-inflammatory activity of essential oils of *D. stramonium* hereby confirmed its traditional use in treating various

inflammatory diseases and may serves as exploitation of *D. stramonium* in treating various inflammatory diseases.

4. Conclusions

To the best of our knowledge, we herein present the first report on the chemical composition and anti-inflammatory property of *D. stramonium* oil growing in Nigeria. The anti-inflammatory property of *D. stramonium* essential oil make their pharmaceutical uses rational and provide a basis for the future work with essential oils.

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