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 activity 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 effects (p<0.001) against 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 anticaner [9] activities. In addition, the anti-inflammatory [10, 11], analgesic, anti diarrhoeal [11], larvicidal [12], pesticidal toxicity [13], antifungal [14], vebriocidal [15] and anticonvulsant [16] potentials of the various extracts of the plant have been reported. The phytochemical compounds isolated from *D. stramonium* such as daturanolone, daturadiol, stigmasterol and sitosterol were shown to possess significant immunostimulatory activity [17]. *D. stramonium* contain variety of alkaloids including atropine, hyoscynine and scopolamine [18], tigloidin, aposcopolamine, apotropine, hyoscyamine N-oxide and scopolamine-N-oxide [18, 19], 6α-ditigloyloxytropane and 7-hydroxyhyoscyamine[2], tropane esters 3-(3′-acetoxytropanyloxy)tropane and 3-(2′-hydroxytropanyloxy)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 °C and 12h light dark cycle); had free access to standard pellet feed and enough drinking water. Ethnic 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 °C) 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 70-240 °C at the rate of 5 °C/min. The ion source was set at 240 °C 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]

\[
% \text{Inhibition of edema} = 100 \times \left( \frac{V_c - V_t}{V_c} \right)
\]

Where, \(V_c\) =Mean paw edema of control group

\(V_t\) =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.

\(~22~\)
Other significant compounds include 6,10,14-trimethyl-2-pentadecanone (9.5%), levomenthol (6.1%), (E)-β-ionone (4.3%), octadecamethylycyclononasiloxane (2.5%) and 13-apo-β-carotene (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 constituents in the seed oil. 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

<table>
<thead>
<tr>
<th>Compounds a</th>
<th>RI b</th>
<th>RI c</th>
<th>Seed</th>
<th>Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetramethylethylene</td>
<td>630</td>
<td>630</td>
<td>3.3</td>
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<tr>
<td>2-Ethyl-4-methyl-1H-pyrrole</td>
<td>982</td>
<td>984</td>
<td>1.9</td>
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<td>Linalool</td>
<td>1100</td>
<td>1095</td>
<td>0.1</td>
<td>-</td>
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<tr>
<td>Levomenthol</td>
<td>1174</td>
<td>1172</td>
<td>-</td>
<td>6.1</td>
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<tr>
<td>4,8-Dimethyl-3,8-dien-2-one</td>
<td>1245</td>
<td>1240</td>
<td>11.2</td>
<td>-</td>
</tr>
<tr>
<td>Citral</td>
<td>1251</td>
<td>1249</td>
<td>26.5</td>
<td>-</td>
</tr>
<tr>
<td>Geraniol</td>
<td>1268</td>
<td>1267</td>
<td>10.5</td>
<td>-</td>
</tr>
<tr>
<td>2-Undecanone</td>
<td>1293</td>
<td>1293</td>
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<tr>
<td>Geranyl acetate</td>
<td>1385</td>
<td>1383</td>
<td>4.7</td>
<td>-</td>
</tr>
<tr>
<td>2-Tridecanone</td>
<td>1493</td>
<td>1494</td>
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<td>-</td>
</tr>
<tr>
<td>(E)-β-Ionone</td>
<td>1498</td>
<td>1496</td>
<td>-</td>
<td>4.3</td>
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<tr>
<td>Sesquirosefuran</td>
<td>1560</td>
<td>1557</td>
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<tr>
<td>(E)-Nerolidol</td>
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<td>1563</td>
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<tr>
<td>Caryophyllene oxide</td>
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<td>Selin-11-en-4-ol</td>
<td>1654</td>
<td>1651</td>
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<td>α-Bisabolol</td>
<td>1690</td>
<td>1685</td>
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<td>Octadecamethylycyclononasiloxane</td>
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<td>1690</td>
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<tr>
<td>α-Farnesol</td>
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<td>1695</td>
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<td>6,10,14-Trimethyl-2-pentadecanone</td>
<td>1848</td>
<td>1847</td>
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<td>(2E,6E)-Farnesyl acetate</td>
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<td>1854</td>
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<td>m-Camphorene</td>
<td>1962</td>
<td>1960</td>
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<tr>
<td>Geranyl linalool</td>
<td>2040</td>
<td>2034</td>
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<td>Phytol</td>
<td>2119</td>
<td>2129</td>
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<td>72.5</td>
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<td>13-apo-β-Carotene</td>
<td>2125</td>
<td>2130</td>
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<tr>
<td>Geranylgeraniol</td>
<td>2204</td>
<td>2201</td>
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</tr>
<tr>
<td>Total</td>
<td>93.1</td>
<td>97.0</td>
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<td>-</td>
</tr>
<tr>
<td>Monoterpenes hydrocarbons</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oxygenated monoterpenes</td>
<td>41.4</td>
<td>10.4</td>
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</tr>
<tr>
<td>Sesquiterpenes hydrocarbons</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oxygenated sesquiterpenes</td>
<td>28.0</td>
<td>9.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diterpenes</td>
<td>4.6</td>
<td>74.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aliphatic ketones</td>
<td>13.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aromatic compounds</td>
<td>5.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Elution order on HP-5MS column; Retention indices on HP-5MS column; Literature retention indices; - Not identified

### 3.2 Anti-inflammatory activity of the essential oils

Figure 1 illustrated the anti-inflammation activity of essential oils of D. stramonium on 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.

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.

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. 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.

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-inflammatory 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. In addition, leaf ketones also exhibit this property which in this case could be attribute to the 6, 10, 14-trimethylycyclononasiloxane 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 conditions.
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.

5. Acknowledgements
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