



## Study on the Chemical Constituents and Anti-inflammatory Activity of Essential Oil of *Petiveria alliacea* L.

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors IAO and OAL designed the study while author AAO carried out the collection of the plant sample and hydrodistillation procedure supervised by author IAO. Author ONA supervised author AAO in the anti-inflammatory test, then performed the statistical analysis and wrote the protocol. Author OO managed the literature searches. Author IAO wrote the first and final drafts of the manuscript. All authors read and approved the final manuscript.

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### ABSTRACT

**Aims:** To study and report the chemical constituents and anti-inflammatory activity of essential oil of *Petiveria alliacea* L (Phytolaccaceae) from Nigeria.

**Study Design:** The study involves the distillation of essential oil from the leaf of *P. alliacea*, characterization of the chemical constituents of the essential oil and the evaluation of its anti-inflammatory potential.

**Place and Duration of Study:** Leaves of *P. alliacea* were collected at Ijede, Ikorodu, Lagos state, Nigeria, in June 2016. Male Wistar rats were obtained and accommodated at the animal facility of Biochemistry Department Lagos state University, Ojo, Nigeria. The experiment lasted till October 2016.

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**Methodology:** Essential oils were obtained by hydrodistillation of air-dried and pulverized leaves of *P. alliacea*. The distilled colourless oil was analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The anti-inflammatory activity was determined on fresh egg albumins over 4 h by measurement of rat paw edema according to established procedure.

**Results:** Eleven compounds representing 99.1% of the oil content were identified in the essential oil of *P. alliacea*. The main constituents were phytol (56.1%), citronellol (16.0%) and (Z, Z)- $\alpha$ -farnesol (14.6%). In the egg albumin-induced edema, essential oils of *P. alliacea* induced time dependent in paw edema. The anti-inflammatory activity was non-significant ( $P = .05$ ) from the 1st h to the 3rd h after egg albumin injection at 1 mL of 2% dose level while the maximum inhibition ( $P < .001$ ) was observed after 4 h. At this hour, the inhibition was more pronounced than Diclofenac sodium injection.

**Conclusion:** The chemical constituents of *P. alliacea* essential oil were found to differ from previous studies. The potent anti-inflammatory activity of essential oils of *P. alliacea* may provide further information on the biological uses of *P. alliacea*.

**Keywords:** *Petiveria alliacea*; hydrodistillation; phytol; citronellol;  $\alpha$ -farnesol; anti-inflammatory assay.

## 1. INTRODUCTION

*Petiveria alliacea* is a species of flowering plant in the family, Phytolaccaceae. It is a deeply rooted herbaceous perennial shrub growing up to 1 m in height and has small greenish piccate flowers. The roots and leaves have a strong acrid, garlic-like odor while the fruits are narrowly oblong 6-8 mm long. In folk medicine, *P. alliacea*, is used to treat a wide variety of disorders [1]. The cytotoxicity [2], hyperglycaemic [3], antimicrobial [4,5], immunomodulatory [6] and antiviral [7] activities of various extracts of *P. alliacea* have been reported. In addition, extracts of *P. alliacea* have demonstrated acaricidal [8], antinociceptive [9], insecticidal [10,11], anti-inflammatory [12,13], analgesic [12] and antioxidant [13] effects. *Petiveria alliacea* extracts elicited mnemonic effects and improved the learning process in rats [14], protects mice against *Listeria monocytogenes* infection [14], exhibited antitumor [15], anxiolytic [16], antiproliferative [15] and allelopathic [17] properties. *Petiveria alliacea* and its major metabolite dibenzyl trisulfide demonstrate HIV-1 reverse transcriptase inhibitory activity [18].

Dibenzyl trisulfide, a major compound of *P. alliacea* was shown to displayed insecticidal [19], acaricidal [20] and erythrocytic membrane [21] effects. The antifungal and antibacterial sulphur activities of other containing compounds of *P. alliacea* such as dipropyl disulphide, dibenzyl sulphide, dibenzyl disulphide, dibenzyl tetrasulphide, benzylhydroxymethyl sulphide and di(benzyltrithio) methane [19,22] have been reported. (Z)-Thiobenzaldehyde S-oxide, isolated from *P. alliacea* was described as a lachrymatory

principle with antibacterial and antifungal activities [23]. The plant also contained stigmaterol, stigmastenol, stigmastanol, loliolide, 3-hydroxy-5,6-epoxy- $\beta$ -ionone and benzyl- $\beta$ -glucopyranoside.

A crude extract of *P. alliacea* analysed by GC-MS [24] was found to comprised of asarone (26.64%), 2-propenonic acid-3-(4-methoxy phenyl) ester (20.95%), phytol (17.43%),  $\alpha$ -D-glucopyranose-4-O- $\alpha$ -D-galactopyranosyl (14.75%), 2, 6,10, 14-tetramethyl-heptadecane, (7.29%). The main compounds found in the inflorescences oil of *P. alliacea* [25] were benzaldehyde (54.8%), benzyl thiol (20.3%) and dibenzyl disulphide (18.0%). The most abundant compounds in essential oils from the aerial parts [26] were toluenethiol (2.3-23.0%), phytol (6.4-40.0%) dibenzyl disulfide (13.2-35.3%) and benzaldehyde (0.8-31.3%). The essential exhibited both antibacterial and antifungal activities against tested organisms [26]. *Petiveria alliacea* produced oils [27] with the principal components being benzyl alcohol (46.6% in the root oil), carvacrol (50.9% in the leaf oil, 48.3% in the stem oil and 29.7% in the flower oil), (Z)-3-hexenyl benzoate (18.6% in the leaf oil, 9.5% in the stem oil and 30.5% in the flower oil) and dibenzyl disulphide (17.6% in the leaf oil, 23.1% in the stem oil, 15.7% in the flower oil and 19.1% in the root oil). The essential oil showed strong acaricidal activities against *Tetranychus urticae* [27]. Benzaldehyde (48.3%), dibenzyl disulfide (23.3%), dibenzyl trisulfide (9.4%), *cis*- and *trans*-stilbene (8.1%) were the major compounds identified from sample grown in Benin [28]. Benzaldehyde (12.6%-55.1%), benzenemethanol (6.2%-14.5%), undecane (3.2%-14.7%), p-vinyl-

guaiacol 13.6%-24.3%), pentadecane (7.6%-29.4%), heneicosane (tr-32.4%) were described in essential oils from various parts of the plant [29]. Phytol, 4-vinyl-2-methoxy-phenol and hexadecanoic acid were previously characterized from the essential oil [30].

The aim of the present research is to report the chemical compounds identified in the essential oil of *P. alliacea* growing in Nigeria as well as the result of the anti-inflammatory activity. This is in continuation of our extensive study on the chemical constituents and biological potentials of Nigerian plants [31].

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

All chemicals and reagents were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA) and were of analytical grade. Ibuprofen injection (May and Baker) was purchased from Lagos State University Pharmacy.

### 2.2 Animals

Male Wistar rats (8 weeks old, 200-240 g) provided by the animal unit of Biochemistry Department were accommodated in the facility at Lagos state University, Ojo, Nigeria. The animals were kept in metal steel cages, where they had unrestricted supply to water and standard pellet food. They were acclimatized for two weeks (temperature  $23 \pm 2^\circ\text{C}$  and 12 h light dark cycle) before commencement of experiment.

### 2.3 Plant Materials

Fresh leaves of *P. alliacea* were collected at Ijede, Ikorodu, Lagos state, Nigeria, in June 2016. Botanical identification was carried out at the Herbarium, Department of Botany, University of Lagos, Nigeria and recorded under the voucher number (LUH7028). The leaves were air-dried under laboratory shade prior to analysis.

#### 2.3.1 Hydrodistillation of essential oils

Air-dried and pulverized leaves (100.0 g) were subjected to hydrodistillation in a Clevenger-type apparatus for 3 h as described previously [31]. The distilled oils were preserved in a sealed sample tube and stored under refrigeration at  $4^\circ\text{C}$  until analysis.

### 2.4 Analysis of Essential Oils

GC analysis was carried out on a Hewlett Packard Gas Chromatography HP 6820 equipped with FID detector and HP-5MS column (60 m x 0.25 mm id), 0.25  $\mu\text{m}$  film thickness and split ratio of 1:25. The oven temperature was programmed from  $50^\circ\text{C}$  (after 2 min) to  $240^\circ\text{C}$  at  $5^\circ\text{C}/\text{min}$  and the final temperature was held for 10 min. Injection and detector temperatures were  $200^\circ\text{C}$  and  $240^\circ\text{C}$  respectively. Hydrogen was the carrier gas at flow rate of 1 mL/min. 0.5  $\mu\text{L}$  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.

GC-MS analysis of the oils 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 (30 m x 0.25 mm id, film thickness 0.25  $\mu\text{m}$ ). The oven temperature was programmed from  $70$ - $240^\circ\text{C}$  at the rate of  $5^\circ\text{C}/\text{min}$ . The ion source was set at  $240^\circ\text{C}$  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  $\mu\text{L}$  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 as previously described [31].

### 2.5 Anti-inflammatory Test on Egg-albumin Induced Rat Paw Edema

The method for the determination of anti-inflammatory activity by measuring rat paw edema reported previously [31] was used in this study. Wistar rats were assigned to one of 3 groups consisting of 5 animals each as follows: group 1-control (treated with 1 ml NaCl (saline) solution), group 2- standard (treated with with Diclofenac Sodium injection 100 mg/kg, orally), while group 3, treated with 2% essential oil suspension of *P. alliacea* orally (using 10 mL/kg; dose volume to animal weight). The same treatments were repeated on day 2 of the experiment. Prior to day 3 experimentation, animals were starved overnight to allow for proper sample absorption into the blood stream through the stomach cavity and to empty part of

gastrointestinal tract [32]. The drug was administered orally to fill the stomach. The respective doses of drugs were fed using cannula needle. Thirty minutes later afterwards, 1.0 mL of 50% (v/v) of fresh egg albumin was injected subcutaneously into the subplantar surface of the right 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:

$$\% \text{ Inhibition of edema} = 100 \times (V_c - V_t / V_c)$$

Where

$V_c$  = Mean paw edema volume of control group  
 $V_t$  = Mean paw edema volume of treated group

## 2.6 Statistical Analysis

Repeated Measures One way ANOVA Analysis using Tukey's multiple comparisons Test was performed using GraphPad Prism (version 7.02), San Diego CA, 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  $\pm$  standard error of the mean.

## 3. RESULTS AND DISCUSSION

### 3.1 Chemical Constituents of the Essential Oil

A colourless oil was obtained in a yield of 0.80% (w/w), calculated on a dry weight basis, from the hydrodistillation of the air-dried leaves of *P. alliacea*. The identity and percentages of the chemical constituents present in the oil and their retention indices on HP-5MS column could be seen in Table 1.

Eleven compounds representing 99.1% of the oil content were identified. The dominant classes of compounds were diterpenes (56.1%), oxygenated monoterpenes (21.5%) and oxygenated sesquiterpenes (14.6%). The main constituents of the oil were phytol (56.1%), citronellol (16.0%) and (Z,Z)- $\alpha$ -farnesol (14.6%). Other significant compounds of the oil were citronellyl acetate (5.5%) and 3-decanol (3.6%). A comparison of previous results on the essential oil of *P. alliacea* revealed that terpenes are

uncommon constituents of the volatile oils. It could be seen that aromatic compounds [24-25, 28-30], sulphur-containing compounds [25-28], fatty acids [29,30] and aromatic esters [24,27,28] were the main classes of compounds present in previous oil samples. Also, the main terpene compounds such asarone [24] and cavacrol [27] identified in oil samples from India and Brazil respectively were conspicuously absent in the studied oil sample. A noteworthy similar was phytol, the main compound in the essential of Nigeria grown *P. alliacea* has been described to be of significant quantity in some previously investigated oil samples [24,26,30]. Moreover, citronellol and (Z,Z)- $\alpha$ -farnesol identified in large quantity in the Nigerian oil sample were not described previously as significant constituents of *P. alliacea* oil. These variations may be attributed to the differences in climatic and ecological conditions between the points of analysis as well age and plant parts used in oil distillation.

### 3.2 Anti-inflammatory Activity of the Essential Oil

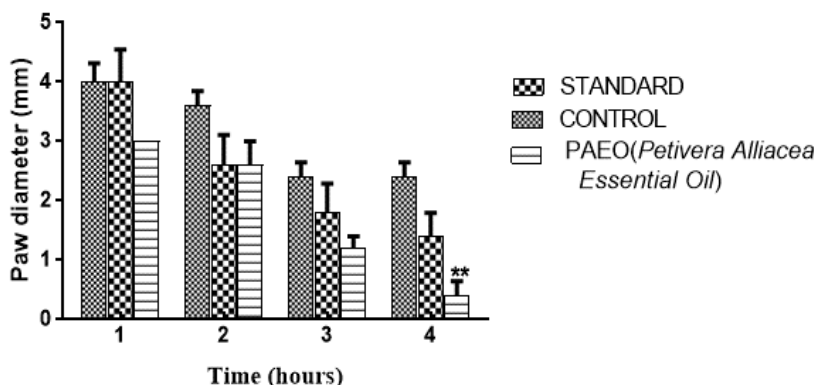
In the egg albumin-induced edema, essential oils of *P. alliacea* induced time dependent in paw edema is summarized in Fig. 1. The anti-inflammatory activity was non-significant ( $P = .05$ ) from the 1<sup>st</sup> h to the 3<sup>rd</sup> h after egg albumin injection at 1 ml of 2% dose level, while maximum inhibition ( $P < .001$ ) was observed after 4 h. At this hour, the inhibition was more pronounced than Diclofenac sodium injection.

The present study is a preliminary assessment of the anti-inflammatory effect of *P. alliacea* collected from the South-Western Nigeria. The egg-albumin model is a time-dependent tri-phasic process. The first state involves the liberation of histamine and serotonin (0–2 h), cytokines, at the 2nd phase (3rd h) and prostaglandin in the 3rd phase (>4 h) [32]. Therefore, *P. alliacea* had been potentially active within the 4<sup>th</sup> hour inhibiting the cyclooxygenase (COX) responsible for prostaglandin synthesis. Plants extracts with such activity are said to be aspirin-like drug [33]. Earlier investigations [12,13] have shown that crude extracts of *P. alliacea* exhibited anti-inflammatory and analgesic activity up to 120 min whereas acetylsalicylic acid did not show further analgesic effect. Using carrageenan model, the root extracts inhibited the build-up of eosinophils and mononuclear cells at the higher dose.

**Table 1. Chemical constituents of essential oil of *P. alliecea***

Compounds <sup>a</sup>	RI (Cal.)	RI (Lit.)	Percentage
Hexanal	810	801	0.1
(2E,4E)-Hexadienal	907	901	tr
$\alpha$ -Thujene	926	921	0.9
$\alpha$ -Pinene	939	932	0.1
3-Decanol	1190	1188	3.6
Citronellol	1225	1223	16.0
Citronelly acetate	1365	1360	5.5
$\beta$ -Elemene	1377	1374	1.3
(Z, Z)- $\alpha$ -Farnesol	1695	1695	14.6
Tetradecanamide	1925	1930	0.9
Phytol	2119	2129	56.1
Total			99.1
Monoterpene hydrocarbons			1.0
Oxygenated monoterpenes			21.5
Sesquiterpene hydrocarbons			1.3
Oxygenated sesquiterpenes			14.6
Diterpenes			56.1
Aliphatic compounds			3.7
Non-terpenes			0.9

<sup>a</sup> Elution order on HP-5MS column; RI (Cal.) Retention indices on HP-5MS column; RI (Lit.) Literature retention indices

**Fig. 1. Anti-inflammatory activity of essential oil of *P. alliecea* on egg albumins over 4 h**

\*\* $P < 0.01$ , \* $P = .05$  extract compared to standard

Although the lipophilic dibenzyl trisulphide was suggested to be the compounds eliciting the anti-inflammatory activity of crude extract of the plant [34]. This compound was not identified in the studied oil sample. The present action may be due to the constituents of the essential oil such as phytol which significantly reduced carrageenan-induced paw edema, in a dose-dependent manner, inhibited histamine, serotonin, bradykinin and PGE2 induced paw edema [35]. Previous results suggested that farnesol supplementation may be beneficial to improve the Th2-skewed allergic asthmatic inflammation [36] while the antinociceptive and

anti-inflammatory properties of citronellol in rodents have been documented [37]. It should be noted that several essential oils have exhibited anti-inflammatory activity on a dose-dependent basis [38] and time-dependent basis [31,39].

#### 4. CONCLUSION

This study revealed that phytol was the major constituents of the essential oil of *P. alliecea* analysed from Nigeria. In addition, the potent anti-inflammatory activity of the essential oil may be attributed to its high content of citronellol, (Z,Z)- $\alpha$ -farnesol and phytol.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

Ethical clearance certificate was obtained from the Research Ethical Clearance Committee (RECC) of the University (Approval no: 013/2016/LASU/BCH).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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