Chemical Components and Antimicrobial Activity of Essential Oils of Petiveria alliacea Leaves Extracts

Ayodele O. Olomieja, Gbenga E. Jolayemi, Fisayo E. Owolabi, Raphael C. Mordi*

Department of Chemistry, Covenant University, Canaan Land, Km 10 Idiroko Road, Ota 111242 Ogun State, Nigeria.
Department of Chemistry, Chrisland University, Km 5, Ajebo Road, Abeokuta, 110222, Ogun State, Nigeria

**Abstract**

A steam distillation technique was employed to obtain oil from the leaf of *P. alliacea*. The oil obtained was subjected to GC/MS analysis to determine the chemical components, which showed the presence of sulphur heterocyclic compounds, dibenzyl disulphide, dibenzyl trisulphide, coumarins, benzaldehyde, benzenecarbothioic acid, tetrathiocane, 1,2,5,6-tetrathiocane and 1,2,3-trithiolane as well as benzenecarbothioic acid (B) that have not been reported previously as components in the crude extracts of *Petiveria alliacea*. The crude extracts showed antimicrobial activity on the following microorganisms: *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Rhizopus sp.*, *Aspergillus niger*. The tests showed that the extract was most effective at limiting the growth of *Salmonella typhi* and the *Rhizopus sp.* (MIC 3.125 μg/mL and MBC value of 6.25 μg/mL). The ethanol extract using the Soxhlet technique was the most effective on *Staphylococcus aureus*, *Escherichia coli*, *Rhizopus sp.*, and *Aspergillus niger* (MIC 3.125 μg/mL).

1. Introduction

Medicinal plants are of great importance to the health of individuals and communities. *Petiveria alliacea* is one such medicinal plant that has been used in different parts of the world, with numerous bio-active compounds (Cseke et al. 2006). The leaf, stem and root decoctions of *Petiveria alliacea* have been employed as a diuretic, antispasmodic agents in traditional medicine and the treatment of cancer, diabetes, nervous disorders, respiratory and pulmonary infections, and as antirheumatic, antifungal, anti-HIV agents, and to enhance memory (Silva et al. 2015). As a result of these uses, this plant has the potential to be applied in the treatment of several ailments (de A Neves et al. 2011; Gomes et al. 2005; Kerdudo et al. 2015; Lopes-Martins et al. 2002; Lowe et al. 2015; Luz et al. 2016).

Some important and interesting compounds have been identified in the extracts from different parts of the plant, including phytol, dibenzyl trisulphide, coumarins, benzaldehyde, benzoic acid, isoarborinol, fredelinol, pinitol and allantonin have been detected in the extracts of various parts of *P. alliacea* (Ayedoun et al. 1998; Castellar et al. 2014; de A Neves et al. 2011; de Andrade et al. 2012; Gomes et al. 2005; Kerdudo et al. 2015; Kim et al. 2006; Lopes-Martins et al. 2002; Oluwa et al. 2017; Randle et al. 2018; Sathiyanabal et al. 2014; Silva et al. 2018; Zavala-Ocampo et al. 2017).

2. Material and methods

Fresh leaves of *P. alliacea* were collected from Iju town in Ado-Odo/Ota local government area of Ogun State. The plant was identified by Dr. Jacob Popoola, Department of Biological Sciences, Covenant University, Ota, and authenticated at the Forestry Research Institute of Nigeria (FRIN). A voucher specimen with the taxonomic identification FHI number (FHI 112438) was deposited in the herbarium of University of Ibadan, Ibadan. The leaves were separated and chopped into small pieces, which were weighed, then first washed with tap water and then with distilled water. The leaves were then allowed to dry at room temperature over three (3) days, after which 480 g of the dried leaves were stuffed into a 1000-mL two-necked flask and 500 mL distilled water was added. The flask was placed on a 1-L heating mantle and steam distillation was carried out over 4 h. The distillate was poured into the separating funnel and 40 mL of diethyl ether was added, the mixture was shaken. Two layers formed, the upper ether layer was then drained off into a 50-mL beaker and then concentrated on a rotary evaporator to obtain the essential oil.

The crude extracts were obtained from the leaves of *P. alliacea* by two different extraction methods namely, cold maceration and Soxhlet extraction (exhaustive and successive) using hexane and ethanol as solvents. For example a total of 128.78 g powdered leaves samples was weighed and about 400 g portions of the leaves samples were placed in the thimble; 1.2 L hexane was measured and poured into the round bottom flask. The Soxhlet apparatus was set up then placed on the heating mantle. The procedure was carried out exhaustively until the

*Corresponding author: Raphael.mordi@covenantuniversity.edu.ng  https://doi.org/10.36547/ae.2020.2.347-50*
extracting solvent was colourless. Further exhaustive extraction was carried out using ethanol solvent on the same leaves samples after the hexane solvent was allowed to dry. The extraction process was handled the same way as the hexane extraction.

For the cold extraction, 1 kg of the leaves sample of *P. alliacea* was weighed and placed in two separate tanks (one of hexane and one of ethanol). The tanks were then covered and left to soak for about 14 days. At the end of the two weeks the samples were decanted and rotary evaporator was used to get the crude extract out.

The antimicrobial analysis was conducted using the Micro-titre technique for the determination of antimicrobial activity using an indicator. Standard micro-tube dilution bio-assay of 96-well micro-titre plates was used to determine the end point, which was taken as the minimum inhibitory concentration (MIC) of the extract samples against the microorganisms. To each of well, 100 µL of sterile 1% glucose peptone water is placed. To well number 1, 100 µL of extract sample was placed and a serial doubling dilution was used to prepare the samples from well number 2 to well number 10. 100 µL broth culture of 0.5 McFarland turbidity identified bacteria was added to all the dilution ranged from well number 1 to well number 10. Overnight broth culture of 100 µL was placed in well number 11 and 100 µL of 1% sterile glucose peptone water was added to serve as control while 100 µL of sterilised 1% glucose peptone was added to 100 µL sterile water in well 12 (blank). The plate was incubated at 37°C for 24 hours. After incubation, 10 µL of phenol red solution (0.025%) was added to each well and colour changes were detected to determine the MIC.

Gas chromatography-mass spectrometry analysis was carried out on the essential oil on GCMS-QP2010SE SHIMADZU instrument. The conditions for the GC analysis are as detailed here.

Column oven temperature was set at 60.0°C; injection temperature was 250.0°C; injection mode was Split with a split ratio of 1:1. The temperature programme was 60.0°C for 2 min then increased at the rate of 13.0°C/min to 260.0°C and held for 2 min. The flow control velocity was linear at 46.3 cm/s and the column flow rate was 3.22 mL/min. The injection quantity was 1 µL. For the mass spectrometry, the ion source temperature was 230.0°C. The solvent cut off time was 4.50 min, with a relative detector mode set at 1.38 kV and threshold value of 2000. The acquisition scan speed was set at 1428.

### 3. Results and discussion

The percentage yield of the crude extract from cold maceration in hexane and ethanol was 11.02% and 8.75% respectively while the Soxhlet extraction it was 54.75% and 40.42% in hexane and ethanol, respectively. The cold extraction yielded less extract compared to Soxhlet extraction. It was also noted that the use of hexane solvent gave better yield compared to that of using ethanol as solvent.

In Table 1, the results of the antimicrobial study are presented. The extract had effects at different concentrations on the test organisms. The study showed that the hexane and ethanol extracts form cold extraction were most effective at limiting the growth of *Salmonella typhi* and the *Rhizopus sp.* respectively with an MIC value of 3.125 µg/mL and MBC value of 6.25 µg/mL. The ethanol extract using Soxhlet extraction technique was most effective at MIC value of 3.125 µg/mL on *Staphylococcus aureus*, *Escherichia coli*, *Rhizopus sp.*, and *Aspergillus niger*. It was also found that the hexane extract with the Soxhlet extraction was effective in inhibiting the growth of *E. coli* and *Rhizopus sp* and the hexane extract inhibited the growth of *S. typhi* microorganisms all at a MIC value of 3.125 µg/mL. Similar results on the effectiveness of the leaf extracts of *P. alliacea* on some of these organisms were reported by Silva et al (2018). They also reported that the hexane extract was more effective at inhibiting the growth of some these organisms than the polar ethanol extract (Silva et al, 2018).

Table 2 shows the retention times and names of compounds identified from the chromatogram, Figure 1. The compounds are numbered in the chromatogram based on the similarity index of greater than 85%. The components identified here made up to 71% of the total distillate. Crude extract from the leaf of *P. alliacea* showed antioxidant activity (Olomieja, 2020), confirming the antioxidant activity reported by other researchers as well as corroborating the antioxidant activity of some sulphur containing compounds.

| Table 1. MIC assay against test microorganisms *P. alliacea* leaves extracts |
|-----------------|-----------|-----------|-----------|-----------|-----------|-----------|
| Organism | *S. typhi* | *S. aureus* | *B. subtilis* | *E. coli* | *Rhizopus sp.* | *A. niger* |
| Cold maceration | 3.12 | 12.5 | 12.5 | 12.5 | 12.5 | 6.25 |
| Hexane | 12.5 | 6.25 | 12.5 | 6.25 | 3.12 | 6.25 |
| Ethanol | 6.25 | 6.25 | 6.25 | 3.12 | 3.12 | 6.25 |
| Soxhlet | 12.5 | 3.12 | 6.25 | 3.12 | 3.12 | 3.12 |
Table 2. Identification of chemical components of *P. alliacea* essential oils using steam distillation

| S/N | Retention time, t<sub>R</sub> (min) | Similarity Index (%) | Compound Name | Compound Structure | Percent composition |
|-----|----------------------------------|----------------------|---------------|-------------------|--------------------|
| 1   | 6.450                            | 96                   | Benzaldehyde, C<sub>7</sub>H<sub>6</sub>O | ![Benzaldehyde Structure](image) | 3.06               |
| 2   | 7.450                            | 95                   | Benzyl alcohol, C<sub>7</sub>H<sub>8</sub>O | ![Benzyl Alcohol Structure](image) | 3.97               |
| 3   | 8.675                            | 89                   | 1,2,3-trithiolane, C<sub>4</sub>H<sub>6</sub>S<sub>3</sub> | ![1,2,3-trithiolane Structure](image) | 0.58               |
| 4   | 11.617                           | 88                   | 1,2,5-trithiepane, C<sub>4</sub>H<sub>8</sub>S<sub>3</sub> | ![1,2,5-trithiepane Structure](image) | 1.13               |
| 7   | 14.410                           | 86                   | 1,2,5,6-tetrathiocane, C<sub>4</sub>H<sub>8</sub>S<sub>4</sub> | ![1,2,5,6-tetrathiocane Structure](image) | 1.27               |
| 8   | 15.667                           | 87                   | Benzenecarbothioic acid, C<sub>7</sub>H<sub>6</sub>SO | ![Benzenecarbothioic Acid Structure](image) | 2.03               |
| 9   | 16.842                           | 87                   | 2-methyltetraicosane, C<sub>25</sub>H<sub>52</sub> | ![2-methyltetraicosane Structure](image) | 1.26               |
| 10  | 17.517                           | 87                   | (Z)-7-hexadecenal, C<sub>16</sub>H<sub>30</sub>O | ![Z-hexadecenal Structure](image) | 1.65               |
| 11  | 17.567                           | 88                   | 2-hexyldecane-1-ol, C<sub>14</sub>H<sub>26</sub>O | ![2-hexyldecane-1-ol Structure](image) | 1.20               |
| 12  | 18.310                           | 89                   | 2-octyldecane-1-ol, C<sub>18</sub>H<sub>38</sub>O | ![2-octyldecane-1-ol Structure](image) | 0.91               |

Figure 1. Chromatogram of the distillate from the steam distillation of the leaf of *Petiveria alliacea* (the numbers are as shown in Table 1).

Many sulphur compounds have been reported in various plant extracts and several have been identified in the extracts of *P. alliacea* in particular that are biologically active (Kim et al., 2006; Lowe, et al., 2015). We report here for the first time the presence of some ringed-sulphur compounds (heterocycles) (3, 4 and 7) as well as benzenecarbothioic acid (8) as components of *Petiveria alliacea*. These compounds make up more than 65% of the mixture. The mass spectral data for these four (4) compounds are shown here. The values for each compound are shown in decreasing order of abundance. 1,2,3-trithiolane, (3)
Conclusion

With the results reported here, it should be noted that the chemical constituents of *P. alliacea* essential oil obtained by steam distillation differ from the chemical constituents obtained by hydrodistillation reported in previous studies. The presence of three new sulphur heterocyclic compounds and benzenecarbothioic acid as constituents in *P. alliacea* essential oil is reported. These compounds were identified by examination of the mass spectral data. The extracts showed antimicrobial activities against some microorganisms studied here.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Ayedoun, M. A., Moudachirou, M., Sosou, P. V., Garneau, F-X., Gagnon, H. Jean, F-L. (1998). Volatile Constituents of the Root Oil of Petiveria alliacea L. from Benin. Journal of Essential Oil Research, 10(6), 645 – 646. [https://doi.org/10.1080/10412905.1998.9700996](https://doi.org/10.1080/10412905.1998.9700996)

2. Castellar, A., Gagliardi, R. F., Mansur, E., Bizzo, H. R., Souza, A. M., Leitão, S. G. (2014). Volatile constituents from in vitro and ex vitro plants of Petiveria alliacea L. Journal of Essential Oil Research, 26(1), 19 – 23. [https://doi.org/10.1080/10412905.2013.820673](https://doi.org/10.1080/10412905.2013.820673)

3. Cseeke, L. J., Kirakosyan, A., Kaufman, P. B., Warber, S., Duke, J. A., Breiholm, H. L. (2006). Natural Products from Plants. (2nd Ed.). Boca Raton: CRC Press Taylor & Francis Group.

4. de Andrade, T. M., Melo, A. S., Dias, R. G. C., Varela, E. L. P., Oliveira, F. R., Vieira, J. L. F., Andrade, M. A., Baetas, A. C., Monteiro, M. C., Maza, C. S. F. (2012). Potential behavioural and pro-oxidant effects of Petiveria alliacea L. extract in adult rats. Journal of Ethnopharmacology, 143(2), 604 – 610. [https://doi.org/10.1016/j.jep.2012.07.020](https://doi.org/10.1016/j.jep.2012.07.020)

5. de A. Neves, I. L. da Camara, C. A. G., de Oliveira, J. C. S., de Almeida, A. V. (2011). Acaridical Activity and Essential Oil Composition of Petiveria alliacea L. from Pernambuco (Northeast Brazil). Journal of Essential oil Research, 23(1), 23 – 26. [https://doi.org/10.1080/10412905.2011.9700426](https://doi.org/10.1080/10412905.2011.9700426)

6. Gomes, P. B., Oliveira, M. M., Nogueira, C. R., Noronha, E. C., Carneiro, L. M., Bezerra, J. N., Neto, M. A., Vasconcelos, S. M., Fontes, M. M., Viana, G. S., de Sousa, F. C. (2005). Study of antimycobacterial effect of isolated fractions from Petiveria alliacea L. (tips) in mice. Biol Pharm Bull., 28(1), 42 – 46. [https://doi.org/10.1248/bpb.28.42](https://doi.org/10.1248/bpb.28.42)

7. Kerdudo, A., Gonnot, V., Ellong, E. N., Boyer, L., Michel, T., Adenet, S., Rochefort, K., Fernandez, X. (2015). Essential oil composition and biological activities of Petiveria alliacea L. from Martinique. Journal of Essential Oil Research, 186 – 196. [https://doi.org/10.1080/10412905.2015.1014118](https://doi.org/10.1080/10412905.2015.1014118)

8. Kim, S., Kubeck, R., Musah, R. A. (2006). Antibacterial and activity of sulfur-containing compounds from Petiveria alliacea L. Journal of Ethnopharmacology, 104, 188 – 192. [https://doi.org/10.1016/j.jep.2005.08.072](https://doi.org/10.1016/j.jep.2005.08.072)

9. Lopes-Martins, R. A., Pegoraro, D. H., Wosisky, R., Penna, S. C., Sertié, J. A. (2002). The anti-inflammatory and analgesic effects of a crude extract of Petiveria alliacea L. (Phytolaccaceae). Phytomedicine, 9(3), 245 – 248. [https://doi.org/10.1076/phyt.9.3.245-248](https://doi.org/10.1076/phyt.9.3.245-248)

10. Lowe, H. I. C., Toyang, N. J., Heredia, A., Ayeak, K. N. N., Watson, C. T., Bryant, J. (2015). Petiveria alliacea L. (Guinea Hen Weed) and Its Major Metabolite Dibenzyl Trisulfide Demonstrate HIV-1 Reverse Transcriptase Inhibitory Activity. European Journal of Medicinal Plants, 5(1), 88 – 94. [https://doi.org/10.9734/EJEMP/2015/12064](https://doi.org/10.9734/EJEMP/2015/12064)

11. Luz, D. A., Pinheiro, A. M., Silva, M. L., Monteiro, M. C., Prediger, R. D., Maia, C. S. F., Fontes-Júnior, E. A. (2016). Ethnobotany, phytochemistry and neuropharmacological effects of Petiveria alliacea L. (Phytolaccaceae): A review. Journal of Ethnopharmacology, 185, 182 – 201. [https://doi.org/10.1016/j.jep.2016.02.053](https://doi.org/10.1016/j.jep.2016.02.053)

12. Olomieja, A. O. (2020). Phytochemical constituents, antimicrobial and antioxidant properties of Petiveria alliacea. [Master’s dissertation]. Ota, Ogun State: Covenant University.

13. Oluwa, A. A., Avoso, O. N., Omokorede, O., Ogunwande, I. A., Lawal, O. A. (2017). Study on the Chemical Constituents and Anti-inflammatory Activity of Essential Oil of Petiveria alliacea L. Journal of Pharmaceutical Research International, 15(1), 1 – 8. [https://doi.org/10.9734/BJPR/2017/51331](https://doi.org/10.9734/BJPR/2017/51331)

14. Randle, M. M., Riley, C. K., Williams, L. A. D., Watson, C. T. (2018). A Systematic Review of the Traditional and Medicinal Uses of Petiveria alliacea L. In The Treatment of Chronic Diseases. Journal of Plant Science and Research, 5(1), 179.

15. Sathiyabalan, G., Packia, L. M., Muthukumarasamy, S., Mohan, V. R. (2014). GC-MS analysis of bioactive components of Petiveria alliacea L. whole plant (Phytolaccaceae). Int J Pharm Res Health Sci., 2(5), 387 – 392.

16. Silva, J. P. B., do Nascimento, S. C. M., Okabe, D. H., Pinto, A. C. G., de Oliveira, F. R., da Paixao, T. P., Queirua, M. L. S., Baetas, A. C., de Andrade, M. A. (2018). Antimicrobial and anticancer potential of Petiveria alliacea L. (Herb to “tame the Master”): A review Phamacogony Review, 12(23), 85 – 93. [https://doi.org/10.4103/phrev.phrev_30_17](https://doi.org/10.4103/phrev.phrev_30_17)

17. Silva, M. L., Luz, D. A., Paixão, T. P., Silva, J. P., Belêm Filho, I. J., Fernandes, L. M., et al. (2015). Petiveria alliacea exerts mnemonic and learning effects on rats. Journal of Ethnopharmacology, 169, 124 129.

18. Zavala-Ocampo, L. M., Aguirre-Hernández, E., Pérez-Hernández, N., Rivera, G., Marchat, L. A., Ramírez-Moreno, E. (2017). Antiinflammatory Activity of Petiveria alliacea Leaves and Their Main Component, Isoarborinol. Journal of Ethnobiology and Biotechnology, 27(8), 1401 – 1408. [https://doi.org/10.4014/jemb.1705.05003](https://doi.org/10.4014/jemb.1705.05003)