PAPER • **OPEN ACCESS**

Antimicrobial and Antioxidant properties of *Petiveria alliacea*

To cite this article: A. O. Olomieja *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **655** 012015

IOP Conf. Series: Earth and Environmental Science **655** (2021) 012015 doi:10.1088/1755-1315/655/1/012015

Antimicrobial and Antioxidant properties of *Petiveria alliacea*

A. O. Olomieja[1*], I. O. Olanrewaju[1], J. I. Ayo-Ajayi[1], G. E. Jolayemi[1], U. O. Daniel[2] and R. C. Mordi[1,3]

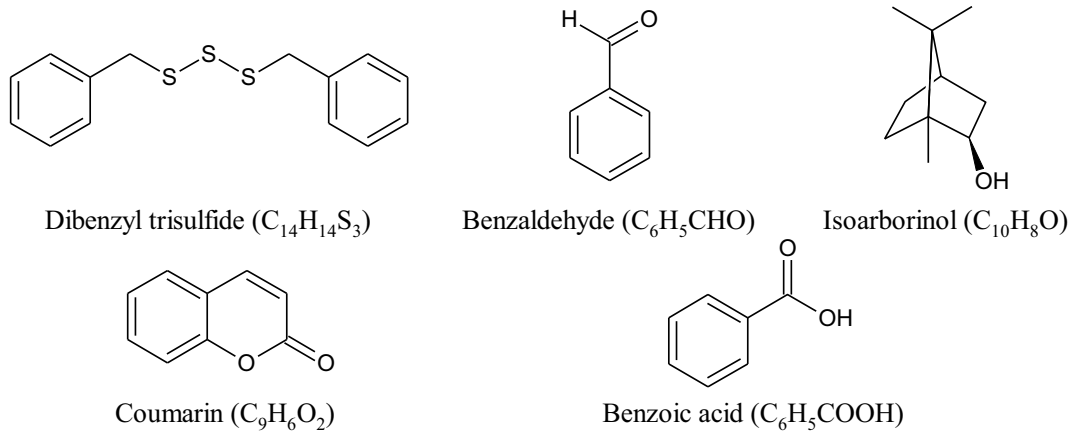
[1]Department of Chemistry, Covenant University, Ota, Ogun State, Nigeria
[2]Department of Biochemistry, Covenant University, Ota, Ogun State, Nigeria
[3]Department of Chemistry, Chrisland University, Ajebo Road, Abeokuta 112422, Ogun State, Nigeria

*Corresponding Author: ayodele.olomiejapgs@stu.cu.edu.ng; +234(0)8143909423

Abstract. The leaf, stem and root decoctions of *Petiveria alliacea* (referred to as Anamu or Apacin (Guatemala), Guinea–Hen weed (English), Awogba or Ojusaju (Yoruba), kanunfari (Hausa) and Akwa-ose (Igbo) have been used as a diuretic, antispasmodic, sedative, analgesic and anti-inflammatory agents in traditional medicine. Cold maceration and Soxhlet extraction techniques were used to obtain oils from the leaf, stem and root of *P. alliacea*. The oils were analysed for their antimicrobial properties using brothmicrodilution technique while their antioxidant properties were determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) tests.The antimicrobial tests on the following micro-organisms *Salmonella typhi, Staphylococcus aureus, Bacillus subtilis, Escherichia coli,*as well as the anti-fungi test on *Rhizopus sp*., and *Aspergillus niger*, showed that the hexane and ethanol cold leaf extracts were most effective in limiting the growth of *Salmonella typhi* with minimum inhibitory concentration (MIC)value of 3.125 µg/mL and minimum bactericidal concentration (MBC)value of 6.25 µg/mL. The ethanol leaf extracts using Soxhlet extraction technique were most effective at MIC value of 3.125 µg/mL on *Staphylococcus aureus*, *Escherichia coli*. Likewise, the hexane and ethanolcold leaf extracts were most effective in limiting the growth of *Rhizopus sp*. and *Aspergillus niger* with MIC value of 3.125 µg/mL and minimum fungicidal concentration (MFC) value of 6.25 µg/mL.
Keywords: *Petiveria alliacea*, crude extracts, antioxidant activity, antimicrobial activity

1. Introduction

Medicinal plants are of great importance to the health of individuals and communities [1].The use of plants as medicines involves the isolation of active compounds from plant components. Man has used plant-based medicines in the form of crude drugs, tinctures, teas, poultices, powders, and other herbal formulations for centuries[2].Indigenous knowledge of plant properties and activities are passed down from generation to generation in various parts of the world, and this knowledge has significantly contributed to the development of different traditional systems of medicine. *Petiveria alliacea* is such a plant that has been used in different parts of the world, with numerous bio-active compounds [3]. *P. alliacea* is known as Guinea–Hen weed in English, it is called Awogba or Ojusaju by the Yoruba speaking people of South-west Nigeria, kanunfari in Hausa, Northern part of Nigeria and Akwa-Ose in Igbo, South–Eastern Nigeria. *Petiveria alliacea* is used in herbal medicine in South Western Nigeria for the treatment of various ailments such as diabetes, arthritis, toothache, skin infection etc.Oluwaet alfound that phytol was the principal constituent of the essential oil of *P. alliacea* [4]. Some other relevant and interesting compounds that have been found to be present in *P.alliacea extracts*, are shown in Figure 1 below. The objective of the study was to extract and identify the components of the plant parts by GCMS analysis and in this paper we present the results of the antimicrobial and antioxidant properties of the extracts.



Dibenzyl trisulfide ($C_{14}H_{14}S_3$) Benzaldehyde (C_6H_5CHO) Isoarborinol ($C_{10}H_8O$)

Coumarin ($C_9H_6O_2$) Benzoic acid (C_6H_5COOH)

Fig 1: Some of the compounds that have been identified from the extracts of *P. alliacea*

2. Materials and Method

All chemical compounds were obtained from commercial sources and were provided by the Department of Chemistry, Covenant University. Organic solvents used in the extraction were Analytical grade Ethanol, 99.5% purity and *n*-Hexane, 99% purity.

The Antioxidant analysis was ran with UV-VIS spectrophotometer, while the Antimicrobial analysis was done using bio–assay of 96 wells micro- titre plates. Soxhlet extractor, Distillation Apparatus, Oven for drying glass wares, Heating mantle**.**

2.1 Plant

The whole plant of *P. alliacea* was collected from Iju town in the Ado–Odo/Ota Local Government Area of Ogun State and authenticated at the Forest Herbarium, Ibadan with the taxonomic identification FHI number 112438. The plant was washed and separated into its different parts (the leaf, the root and the stem) and then dried at room temperature for 30 days. The dried plant parts were ground into small size particles, weighed and kept in a plastic container until required for further work.

2.2 Extraction

The crude extracts were obtained from the leaf, stem and root of *P. alliacea* by cold maceration and Soxhlet extraction technique (exhaustive and successive) using hexane and ethanol as solvents. For example a total of 1287.87g powdered sample (leaf) was weighed and portions of the leaf samples were placed in the thimble; 1.2 L hexane was measured and poured into the round bottomed flask. The Soxhlet apparatus was set up then placed on the heating mantle which was then turned on. The procedure was carried out exhaustively until the extracting solvent was colourless. The procedure as described above was also used in the extraction of the specified amounts of the root and stem parts of the plant. Further exhaustive extraction was carried out on dried samples (leaf, stem and root) using ethanol as solvent. The extraction process was handled exactly the same way as the hexane extraction.

For the cold extraction, 1 kg each of the (root, stem and leaf) sample of *P. alliacea* was weighed and placed in six different tanks (three of hexane and three 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.

2.3 Phytochemical Analysis

The phytochemical analyses of extracts were carried out for the presence of quinones, tannins, flavonoids, phenols, coumarins, saponins, sugar and glycosides using standard qualitative methods [6].

2.4 Antimicrobial Activity

The broth microdilution technique for antimicrobial activity determination was used withphenol red indicator. TheMIC values of the effect of the crude extracts on the following organisms *Salmonella typhi, Staphylococcus aureus Bacillus subtilis, Escherichia coli, Rhizopus sp., Aspergillus niger,* were determined.The indicator end point, which is the MIC of the extract samples against the microorganism strains, was determined using standard micro-tube dilution bio-assay of 96 wells micro-titre plates.

To each well, 100µLof sterile 1% glucose peptone water was placed in each well in horizontal direction, then doubling serial dilution of the extract was made from 100 µL, 50µL, 25 µL, 12.5µL, 6.25 µL and 3.125 µL and were respectively placed in the wells in the vertical direction.Equal volume of 100µL broth culture of 0.5 McFarland turbid identified bacteria was added to all the dilution with the exception of two wells, which served as control.The plate was incubated at 37^{o}C for 24 hours. After incubation, 10 µL of phenol red solution (0.025%) was added to the contents in the 96 wells [7].

2.5 Antioxidant Activity

Antioxidant activity of the crude extracts were determined by their effect on DPPH and compared against ascorbic acid standard.Spectroscopic analyses were carried out at 517nm.

One gram each of the leaf, stem and root of the crude extract was weighed and then transferred into different 100mLconical flasks. Enough methanol was then added to each conical flask to dissolve the extract. Each solution of each extract was then transferred to three different 25 mL volumetric flasks; made up to mark with methanoland kept in the dark for further analysis. For further analytical work, for example for the leaf extract, 1 mL, 2 mL, 3 mL, 4 mL and 5 mL of extract solution was taken and placed in five different test tubes respectively and adding enough methanol to a total of 5 mL in each test tube. After mixing, 1mL of each solution was taken and transferred to another set 5 different test tubes. To these new test tubes, 3 mL of the prepared DPPH was then added. These test tubes were kept in the dark for 30 minutes after which the spectroscopy analysis was undertaken. The same procedure was used for the preparation of extracts from the stem and root parts of the plant [8].

Ascorbic acid (0.04g) was also weighedinto a 100 mL beaker, dissolved in distilled water and transferred to a 100 mL volumetric flask and made up to mark. Solutions for analytical work were prepared in the same way as the extract samples.

To a 100 mL volumetric flask, 0.004g of DPPH was added. Methanol (100 mL) was measuredinto the volumetric flask containing the sample. The content in the flask was then wrapped with aluminium foil to prevent light penetration into the sample, then kept in the dark for further analysis [8].

3. Results and Discussion

3.1 *Percentage Yield*

Table 1 shows the percentage yield of the crude extracts.

From Table 1, it was observed that cold extraction gave a poorer percentage yield compared to the Soxhlet extraction. It was also observed that the extract from the leaf gave better yield as has been previously reported [5]. Comparing the two methods of extraction we found that the use of hexane as solvent for extraction gave better yield than the use of ethanol as solvent.

3.2 Phytochemical Analysis

The results of the phytochemical screening of the oils are presented in Tables 2a-2b. All the extracts gave positive results with the test reagents.Using the Soxhlet extraction method, it was observed that all the phytochemicals tested for were found in the hexane leaf extract except tannins, flavonoids and glycosides. Flavonoids and quinones were the only phytochemicals found in the ethanol root extract. With hexane as the extracting solvent, only quinones, tannin and coumarin were found in the root extract. With cold extraction, quinones were found in all the extracts for both hexane and ethanol as solvents. No phenol, saponins, glycosides and tannins were found in the ethanol extracts. No flavonoids were found in the hexane extracts [6]

Table 1: The percentage yield of the crude extract from *P. alliacea*

	Cold extraction						Soxhlet extraction					
	Hexane			Ethanol			Hexane			Ethanol		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
Starting mass of plant part/g	774.4	1932.0	1039.3	714.9	718.8	1003.3	1287.9	1598.4	1459.6	1287.9	1598.4	1459.6
Mass of crude extract/g	85.31	193.78	98.777	62.308	60.970	120.00	705.12	920.18	600.25	520.14	610.40	518.32
Percentage yield/%	11.02	10.03	9.50	8.75	8.48	11.96	54.75	57.57	41.13	40.42	38.19	39.62

Table 2a: Results of Phytochemical screening of the crude extracts from *P. alliacea* by Cold Extraction.

Phytochemical	Extraction solvent/Plant part				
	Hexane			Ethanol	
	Leaf	Stem	Root	Leaf	Stem
Quinone	–	++	++	+++	+++
Tannin	–	+	+	–	–
Flavonoid	–	–	–	+	++
Phenol	++	+	+	–	–
Coumarin	++	+++	++	+	–
Saponin	+	–	–	–	–
Sugar	–	+	–	–	+
Glycoside	–	–	–	–	–

3.3 Antimicrobial Activity:

In Table 3, the results of the antimicrobial study are presented. We see that all the extracts had effect at different concentrations on the test organisms under study. The study showed that the cold hexane leaf extract and cold ethanol leaf extract 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 MFC value of 6.25µg/mL.The ethanol leaf extracts using Soxhlet extraction technique were most effective at MIC value of 3.125 µg/mL on *Staphylococcus aureus, Escherichia coli, Rhizopus sp., and Aspergillus niger*. It also found that the hexane leaf extract with the Soxhlet extraction technique was effective on inhibiting the growth of *E. coli* and *Rhizopussp*. and the hexane extract inhibited the growth of *S. typhi* micro-organisms all at an MIC value of 3.125 µg/mL.

Table 2b: Results for Phytochemical screening of the crude extracts from*P. alliacea*by Soxhlet Extraction

Phytochemical	Extraction solvent/Plant part				
	Hexane			Ethanol	
	Leaf	Stem	Root	Leaf	Root
Quinone	++	++	++	++	++
Tannin	–	+	+	–	–
Flavonoid	–	–	–	–	++
Phenol	++	+	–	+	–
Coumarin	++	+	++	–	–
Saponin	+	–	–	–	–
Sugar	++	–	–	++	–
Glycoside	–	++	–	–	–

(+) signifies that the substance is present (the greater the number of + sign, the more intense the colour observed suggesting a greater amount of substance in extract;**(–)** signifies that the substance is not present in extract.

Table 3: MIC values of the crude extractsfrom *P. alliacea*against test microorganisms

	Cold extraction					Soxhlet extraction				
	Hexane			ethanol		Hexane			Ethanol	
	Stem	Leaf	Root	Leaf	Stem	Stem	Leaf	Root	Leaf	Root
Organisms	Concentration (μg/mL)									
Salmonella typhi	12.5	3.125	6.25	12.5	12.5	3.125	6.25	12.5	12.5	25.0
Staphylococcus aureus	12.5	12.5	6.25	6.25	6.25	12.5	6.25	50.0	3.125	25.0
Bacillus subtilis	25.0	12.5	12.5	12.5	12.5	12.5	6.25	6.25	6.25	6.25
Escherichia coli	12.5	12.5	6.25	6.25	12.5	6.25	3.125	6.25	3.125	6.25
Rhizopus sp.	6.25	12.5	25.0	3.125	6.25	12.5	3.125	6.25	3.125	6.25
Aspergillus niger	6.25	6.25	12.5	6.25	12.5	6.25	6.25	12.5	3.125	12.5

3.4 Antioxidant Activity

Table 4 and Figures 2 and 3 show the absorbance values and the plots for the absorbance of the crude extracts. We observed that in both extraction techniques leaf extracts were more effective at scavenging DPPH than the stem and root extracts. With both extraction techniques it was observed that with ethanol as solvent for extraction, the scavenging of DPPH was higher than when hexane was the extraction solvent. This observation is not surprising as ethanol is a more polar solvent and it is expected to extract the more polar antioxidant components in the plant

material. This expectation is actually borne out by phytochemical analysis and analyses of the components of the extracts.

Table 4 Absorbance values for the interaction of the extracts with DPPH

	Cold (Hexane)			Cold (Ethanol)		Soxhlet (Hexane)			Soxhlet (Ethanol)		
						Absorbance					
Concentration	Crude extract (Root)	Crude extract (Stem)	Crude extract (Leaf)	Crude extract (Stem)	Crude extract (Leaf)	Crude extract (Root)	Crude extract (Stem)	Crude extract (Leaf)	Crude extract (Root)	Crude Extract (Stem)	Crude extract (Leaf)
80 µg/mL	0.081	0.087	0.105	0.122	0.245	0.080	0.090	0.129	0.096	0.095	0.138
160 µg/mL	0.088	0.089	0.126	0.166	0.239	0.078	0.092	0.145	0.102	0.088	0.188
240 µg/mL	0.087	0.103	0.149	0.182	0.452	0.085	0.095	0.183	0.160	0.094	0.284
320 µg/mL	0.101	0.128	0.172	0.223	0.586	0.086	0.105	0.226	0.133	0.088	0.391
400 µg/mL	0.092	0.092	0.165	0.241	0.754	0.092	0.102	0.260	0.198	0.094	0.364

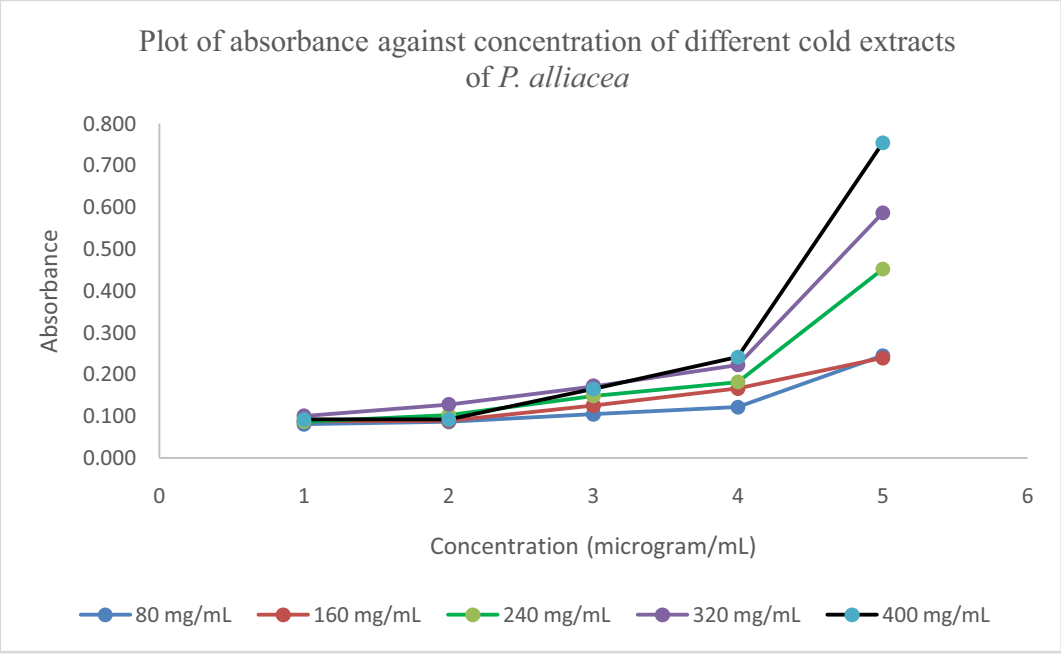


Figure 2: Plot of absorbance against concentration of cold extracts

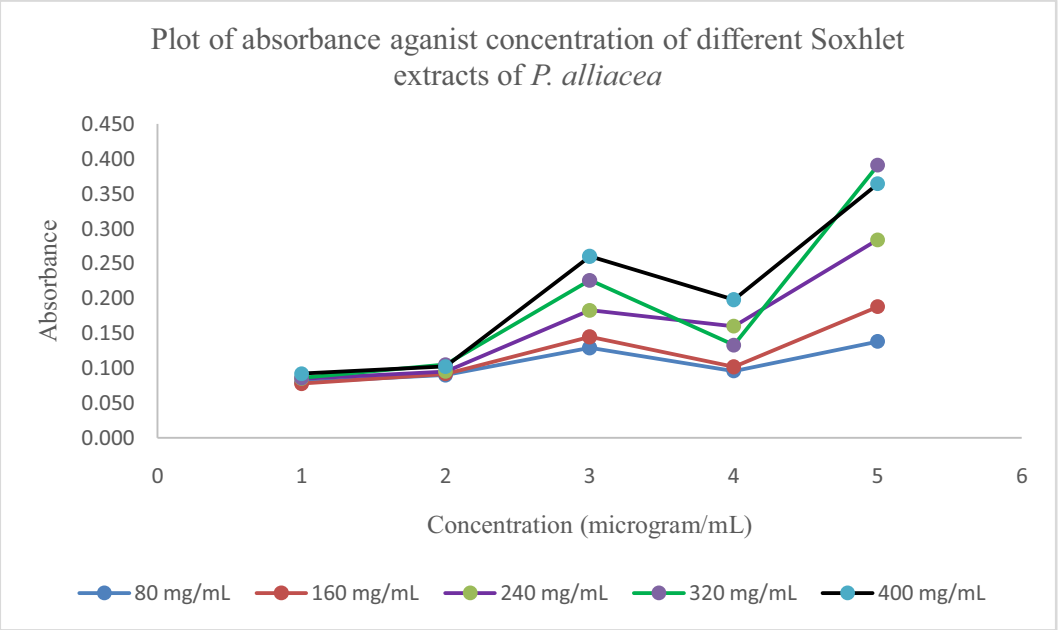


Figure 3: Plot of absorbance against concentration of Soxhlet extracts

4. Conclusion

With the observed facts, it can be concluded that all parts of *P. alliacea* are available for use in treatment of ailments that involve the microorganisms we worked on in this report as well ailments that result from oxidative damage of cells. Therefore, various ways to isolate the active ingredients for drug preparation would be looked into.

References

[1] Edeoga H., Okwu D., Mbaebie B (2005). Phytochemical constituents of some Nigerian medicinal plants, *Afr. J. Biotechnol* 4(7), 685-688.

[2] Dias, D. A., Urban, S., and Roessner, U. (2012). A historical overview of natural products in drug discovery. *Metabolites*, 2(2), 303-336.

[3] Cseke L.J., KirakosyanA., Kaufman P.B., WarberS., Duke J.A., Brielmann H.L (2006). Natural Products from Plants. (2nd Ed.). Boca Raton: CRC Press Taylor & Francis Group.

[4] Oluwa A.A., Avose H.O., Omikorede O., Ogunwande I., Lawal O.A (2017). Study on the chemical constituents and Anti-inflammatory activity of essential oil of *Petiveria alliacea*L. *Br J Pharm Res* 15, 1-8

[5] Silva J., do Nascimento S., Okabe D., Pinto A., de Oliveira F., da Paixao T., Siqueira M., Baeta A., de Andrade M (2018). Antimicrobial and anticancer potential of *Petiveria alliacea* L. (Herb to "Tame the Master"): A review *Pharmacognosy Review* 12(23), 85 – 93.

[6] Drummond, A.J., &Waigh, R.D. (2000). The development of microbiological methods for phytochemical screening. Recent research development in phytochemistry, 4, 143 - 152.

[7] Trease, G.E. and Evans, W.C. (1998). Pharmacology, 11th Edition, Bailliere Tindall Ltd; London, 60 - 75.

[8] Brand-Williams, W.; Cuvelier, M.E.; Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28, 25 - 30.