PAPER • **OPEN ACCESS**

Potency of the Leaf, Stem and Root Extracts of *Petiveria alliacea* Against Selected Microorganisms

To cite this article: A. O. Olomieja *et al* 2021 *IOP Conf. Ser.: Mater. Sci. Eng.* **1107** 012051

POTENCY OF THE LEAF, STEM AND ROOT EXTRACTS OF *Petiveria alliacea* AGAINST SELECTED MICRO-ORGANISMS

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, Chris Land University, Ajebo Road, Abeokuta, Ogun State, Nigeria.

*Corresponding Author; mayormazy@yahoo.com +234(0)8143909423

Abstract:

Infectious diseases have contributed immensely to the rate of death globally in the past decades due to increased resistance to synthetic drugs. Hence, there is an urgent need to find a lasting solution to the problem of drug resistance, through the emergence of alternative medicine. One of such plant with biologically – active components against variety of diseases is *Petiveria alliacea*. It has been used to reduce inflammations and pains and also to eliminate bacteria, fungi, candida and viruses. The potency of the oils obtained from the leaves, stems and roots of this plant through (Soxhlet and Cold extraction methods) were subjected to test against certain micro-organisms, such as: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* as well as the anti-fungi test on *Rhizopus sp*., and *Aspergillus niger.* 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 MBC value of 6.25 µg/mL. The ethanol leaf extracts using Soxhlet technique were most effective at MIC value of 3.125 µg/mL on *Staphylococcus aureus, Escherichia coli, Rhizopus sp., and Aspergillus niger.* 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 ethanol cold 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. The antioxidant property of the extracts was determined by 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay with ascorbic acid as the reference standard.

Key words: Infectious diseases, Synthetic drugs, Potency, Antioxidant, 2,2-Diphenyl-1-picrylhydrazyl

1. Introduction

Microbial infection is a known major challenge to every human, which has led to the loss of lives over the decades. Overcoming this challenge has become more worrisome due to the emergence of drug resistance by microorganisms that cause these illnesses [1]. Studies have revealed that life-threatening infections caused by these microbes are becoming more and this had led to high

rates of death globally. Microbes are known to be causative agents to many diseases both communicable and non-communicable, but the way out to these problems are by the use of alternative medicine [1]. Among the plethora of medicinal plants being currently investigated for their ameliorative properties in this 21st century is *Petiveria alliacea* [2]. *Petiveria alliacea* is called Guinea-hen weed in English, Awogba arun in Yoruba, Akwa –ose in Igbo and kanunfari in Hausa. It is a very important plant in traditional Latin America herbal medicine where it is used as an anti-rheumatic, anti-inflammatory, to treat fever, headache, diabetes, malaria, arthritis, skin allergies, cancer, and to induce abortions and has been used to reduce pains. It has been reported to have been used to eliminate bacteria, fungi, candida, and viruses [3]. In South-West Nigeria, the macerated leaves are generally rubbed on the belly of pregnant women experiencing difficulty in delivering during labour and could stimulate movement in the limbs of paralyzed individuals [4]. It can also be used as an analgesic for muscular pain and to cure rashes and other skin infections. Likewise, For the treatment of colic, rheumatism, cancer, syphilis, colds, fever, bronchitis and asthma [4].

The main compounds from *P. alliacea* include lipids, flavonoids, and triterpenes. Sulphur compounds known as azure derivatives are unique to this species [5]. *P. alliacea* had been found to possess a huge number of bioactive compounds, and such compound includes: Tannins, isoarborinol, isoarborinol cinnamate (Benzyl cinnamate), Isothiocyanate (allyl isothiocyanate), Polyphenol (Ellagic acid). Structures of bioactive compounds extracted from *Petiveria alliacea* is as shown in figure 1 below:

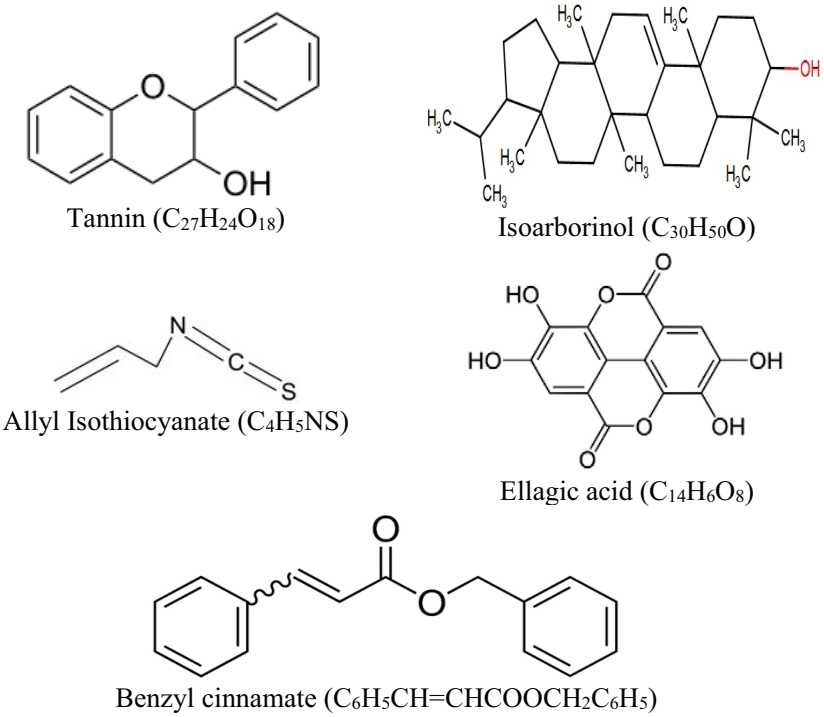


Figure 1: Some bioactive compounds identified from the extracts of *P. alliacea*

2. Materials and Method

Chemical compounds used for the research work were gotten from commercial sources and were provided by the Chemistry Department, Covenant University, Ota. Organic solvents used for the extraction were Reagent grade type hexane (for the exhaustive extractions) and ethanol (for successive extractions).

2.1 Plant

The plant was identified and authenticated at The Forestry Research Institute of Nigeria,(FRIN) Ibadan with Voucher number: 112438.The plant was washed and separated into its different parts (the leaves, roots and stems) and then dried at room temperature for 30 days.

The dried parts were blended into powdery form, then kept in a plastic container.

2.2 Extraction

Extracts from *P. alliacea* were obtained from the leaf, stem and root of the plant by soxhlet extraction method using hexane (done exhaustively) followed by ethanol (carried out successively. Cold maceration technique was also employed, using the same solvent.

For instance, 1300.00g powdered sample of the grinded leaf was weighed and placed in the thimble; 1.3 L hexane was measured and transferred 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 becomes 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 successive extraction was carried out on dried samples (leaf, stem and root) using ethanol as solvent. The extraction process was done the same way as the hexane extraction.

However, for the cold extraction method, 1000.00g 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 two weeks. At the end of the fourteen days, the samples were decanted and rotary evaporator was used to get the crude extract out.

2.3 Phytochemical Analysis

Phytochemical analysis of the extracts was determined for the presence of tannins, flavonoids, phenols, coumarins, saponins, sugar, glycosides and quinones using standard qualitative methods [7].

2.4 Antimicrobial Activity

Standard broth micro-tube dilution bio-assay of 96 wells micro-titre plates was used to determine endpoint, which is the minimum inhibitory concentration (MIC) of the extract samples against the below microorganisms[6]

Salmonella typhi, Staphylococcus aureus, Bacillus subtilis,Escherichia coli, Rhizopus sp., and Aspergillus niger . Each wells contains 100µL of sterile 1% glucose peptone water, 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. 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.

2.5 Antioxidant Activity

Antioxidant activity of the crude extracts were carried out by their effect on 2,2-diphenyl-1-picrylhydrazyl and compared against ascorbic acid standard. Spectroscopic analyses were carried out at 517 nm[8].

One gram each of the leaf, stem and root of the crude extract was weighed and placed in different 100 mL conical flasks. Enough quantity of methanol was 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 methanol and kept in the dark for further analytical work, for example, for the root 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 carried out. The same procedure was used for the preparation of extracts from the leaf and stem parts of the plant [8].

Ascorbic acid (0.04g) was also weighed and poured into 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 measured into the volumetric flask containing the sample. The content in the flask was then wrapped with

aluminum foil to prevent light penetration into the sample, then kept in the dark for further analysis [8].

3. Results and Discussion

3.1 Phytochemical Analysis

All the extracts, either those obtained from the leaf, stem or root gave positive results with the test reagents. In the Soxhlet extraction method (using hexane), it was observed that all the phytochemicals tested for were found in the hexane leaf extract except tannins, flavonoids and glycosides. In the ethanol root extract, flavonoids and quinones were the only phytochemicals found. In the root extract using hexane as the extracting solvent, only quinones, tannin and coumarin were found.

In cold extraction method, using hexane and ethanol (root extracts), 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 1a - 1b shows results for phytochemical screening of the crude extracts of *P. alliacea* by soxhlet and cold Extraction methods

Table 1a: Results of 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	–	++	–	–	–

Table 1b: 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	–	–	–	–	–

(+) 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.

3.2 Antimicrobial Activity:

It was observed that all the extracts had effect at different concentrations on the test organisms under study. The study revealed that the cold hexane leaf extract and cold ethanol leaf extract has higher potency at limiting the growth of *Salmonella typhi* and the *Rhizopus sp*. respectively with an MIC value of 3.125 mg/mL and MFC value of 6.25 mg/mL.

The ethanol leaf extracts using Soxhlet technique has efficacy against the growth of *Staphylococcus aureus, Escherichia coli, Rhizopus sp., and Aspergillus niger* at MIC value of 3.125mg/mL.

The leaf extract using ethanol (soxhlet) was effective against *E. coli and Rhizopus spp* [6]. Colour change were detected to determine the minimum inhibitory concentration (MIC) and the values is as shown in Table 2.

Table 2: MIC values of the crude extracts from *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

3.3 Antioxidant Activity

The result for absorbance values for the interaction of DPPH with Ascorbic acid as well as Percentage DPPH scavenging power of the extracts of *Petiveria alliacea* is as shown in Table 3a – 3b, Table 3c shows the percentage inhibition between DPPH, Ascorbic acid and the crude extract both in Hexane and Ethanol, while figure 2 shows Percentage inhibition of ascorbic acid against the concentration of crude extract from different parts of *P. alliacea* .

Table 3a: Result for absorbance values for interaction of DPPH with Ascorbic acid.

Concentration mg/mL	Absorbance (DDPH + Ethanol)
0.00mg/mL	0.147
0.04 mg/mL	0.1005
0.08 mg/mL	0.0925
0.12 mg/mL	0.0915
0.16 mg/mL	0.0845
0.20 mg/mL	0.0720

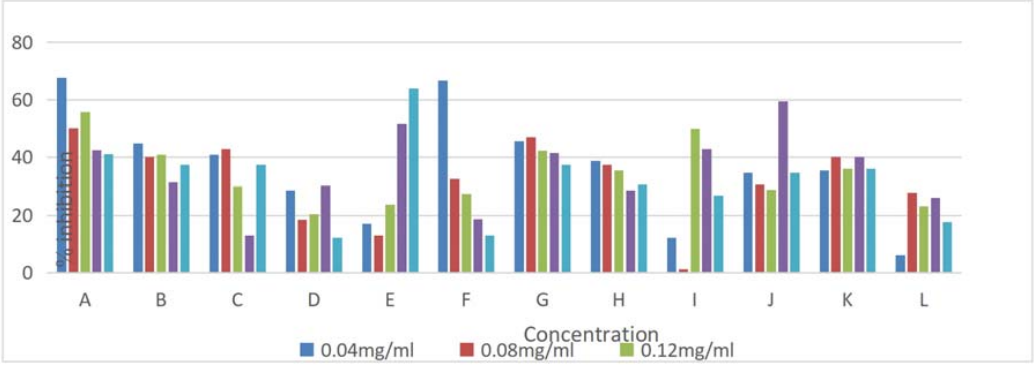
Table 3b: Percentage DPPH scavenging power of the extracts of *Petiveria alliacea*

	Cold (Hexane)			Cold (Ethanol)		Soxhlet (Hexane)			Soxhlet (Ethanol)		
	% DPPH scavenged										
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)
0.04 mg/Ml	0.081	0.087	0.105	0.122	0.245	0.080	0.090	0.129	0.096	0.095	0.138
0.08mg/Ml	0.088	0.089	0.126	0.166	0.239	0.078	0.092	0.145	0.102	0.088	0.188
0.12 mg/mL	0.087	0.103	0.149	0.182	0.452	0.085	0.095	0.183	0.160	0.094	0.284
0.16 mg/Ml	0.101	0.128	0.172	0.223	0.586	0.086	0.105	0.226	0.133	0.088	0.391
0.20 mg/Ml	0.092	0.092	0.165	0.241	0.754	0.092	0.102	0.260	0.198	0.094	0.364

$$\%inhibition = \frac{A(control) - A(sample)}{A(control)} \times 100$$

Table 3c*:* The percentage inhibition for each extract in hexane and ethanol for both cold and soxhlet extraction method.

Conc (mg/mL)	A	B	C	D	E	F	G	H	I	J	K	L
0.04	67.63	44.88	40.81	28.57	17	66.66	45.57	38.77	12.24	34.69	35.37	6.22
0.08	50.07	40.13	42.85	18.36	12.92	32.58	46.93	37.41	1.36	30.61	40.13	27.89
0.12	55.75	40.81	29.93	20.36	23.8	27.48	42.17	35.37	49.87	28.84	36.05	23.19
0.16	42.51	31.29	12.92	30.17	51.7	18.63	41.49	28.57	42.74	59.52	40.13	25.98
0.20	41.02	37.41	37.41	12.24	63.94	12.92	37.41	30.61	26.87	34.69	36.05	17.61



Percentage absorbance for DPPH and Ascorbic acid

Percentage absorbance for crude extract (root) in hexane (cold extraction).
Percentage absorbance for crude extract (stem) in hexane (cold extraction).
Percentage absorbance for crude extract (root) in hexane (soxhlet extraction).
Percentage absorbance for crude extract (stem) in hexane (soxhlet extraction)

A= Percentage absorbance for DPPH and Ascorbic acid.
B= Percentage absorbance for crude extract (Root) in Hexane (Cold Extraction).
C= Percentage absorbance for crude extract (Stem) in Hexane (Cold Extraction).
D= Percentage absorbance for crude extract (Leaf) in Hexane (Cold Extraction).
E= Percentage absorbance for crude extract (Stem) in Ethanol (Cold Extraction).
F = Percentage absorbance for crude extract (Leaf) in Ethanol (Cold Extraction).
G= Percentage absorbance for crude extract (Root) in Hexane (Soxhlet Extraction).
H= Percentage absorbance for crude extract (Stem) in Hexane (Soxhlet Extraction).
I= Percentage absorbance for crude extract (Leaf) in Hexane (Soxhlet Extraction).
J= Percentage absorbance for crude extract (Root) in Ethanol (Soxhlet Extraction).
K= Percentage absorbance for crude extract (Stem) in Ethanol (Soxhlet Extraction).
L = Percentage absorbance for crude extract (Leaf) in Ethanol (Soxhlet Extraction).

It was observed that in both extraction methods, leaf extracts were more effective at scavenging DPPH than the stem and root extracts. This implies that ethanol is a more polar solvent and it is expected to extract the more polar antioxidant components in the plant material

4. Conclusion

With the observed facts, we conclude that
The different parts of *Petiveria alliacea* has potency against the growth of the micro-organisms we worked on in this research work.
Scientific validation of the ethno botanical claim of *P. alliacea* plant was also unraved. We shall, therefore focus on ways of isolating the active ingredients in different parts of the plant for drug preparation.

5. Acknowledgement

The authors acknowledge Covenant University, Ota for her financial support throughout our research work.

References

[1] Lipkin, W. I. (2010). Microbe hunting. Microbiology and Molecular Biology r*eviews.,* 74(3), 363-377.

[2] Pérez-Leal, R., García-Mateos, M., Martínez-Vásquez, M., & Soto-Hernández, M. (2006). "Cytotoxic and antioxidant activity of *Petiveria alliacea* L."*Revista Chapingo. Serie Horticultura.* 12(1), 51-56.

[3] Hernandez, J., Urueña, C., Cifuentes, M., Sandoval, T., & Pombo, L. (2014): A *Petiveria alliacea* standardized fraction induces breast adenocarcinoma cell death by

Modulating glycolytic metabolism. *Journal of Ethno pharmacology*, 153(1), 641- 649.

[4] Oluwa, A., Avoseh, O., Omikorede, O., Ogunwande, I., & Lawal, O. (2017): Study on the Chemical constituents and Anti-inflammatory activity of essential oil of *Petiveria alliacea L. Journal of Pharmaceutical Research international*, 15, 1-8

[5] Fernández Hurtado, D. (2016). Effect of the aqueous extract of the Anamú (*Petiveria alliacea L.)* on Rhizobium spp (Doctoral dissertation, Central University "Marta Abreu" of Las Villas. Faculty of Agricultural Sciences. Department of Agronomy).

[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. & 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 Science and Technology. 28, 25 - 30.

[9] Gentry, A. H. (Ed.). (1993). Four Neotropical Rainforests. Yale University Press. Pp 10 – 15.

[10] 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.

[11] Olomieja, A. O. (2020). Phytochemical constituents, antimicrobial and antioxidant properties of *Petiveria alliacea*. [Master's dissertation]. Covenant University, Ota, Ogun State, Nigeria.

[12] Akinduti, P.A., Motayo, B., Idowu, O.M., Isibor, P.O., Olasehinde, G.I., Obafemi, Y.D., Ughoko, H.U., Oyewale, J.O., Oluwadun, A., & Adeyemi, G.A. (2019). Suitability of Spectrophotometric assay for determination of honey microbial inhibition. *Journal of Physics*: 1299(2019), 012131 doi:10.1088/1742-6596/1299/1/012131