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Antifungal, Antibacterial and Phytochemical Properties of Petiveria Alliacea Plant from Iwo, Nigeria

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Abstract *Petiveria* alliacea plant has been a medicinal plant for decades and has been used both in the traditional and modern ways over these periods. This article highlights part of its medicinal properties particularly, antimicrobial and phytochemicals. Antibacterial and antifungal assay was carried out on both the leaves and root extracts and it was discovered that, for the antibacterial, the methanolic extracts of roots are more effective while the chloroform extracts of leaves are more efficacious. The leaves extracts however proved to be a better antifungal than the root extracts. The following phytochemicals (alkaloids, saponins, flavonoids, tannins, terpenoids and steroids) were analyses in five different extracts (aqueous, methanolic and chloroform extracts of roots as well as chloroform and methanolic extracts of leaves). The results showed that aqueous extracts is more useful medicinally for it contains more phytochemicals than other extracts.

Keywords Petiveria, antibacterial, antifungal, phytochemical

1. Introduction

Petiveria alliacea popularly known as Guinea hen weed in English and '*anamu'* in most part of South America countries where is very common and also referred to as '*awogbaarun'* meaning 'heals many ailments' or '*arunyanyan'* meaning 'smells shaply' in the southern part of Nigeria where it is also in abundance [1]. It is a perennial short plant with a deep tap root and height about 0.5-1.5 meters.

Figure 1: Petiveria alliacea leaves

Petiveria alliacea has been a very useful medicinal plant which has been used by herbalist for curing several ailments over the decades and several researchers have isolated several compounds from different parts of the plants, which ranged from coumarins to sulphoxides and many other classes of organic compounds particularly compounds of sulphur. In fact, it is believed in some part of Southern Nigeria (particularly among villagers) that the plant cures so many ailments which really cannot be counted and that led to naming it 'awogbaarun' as mentioned earlier.

Petiveria alliacea has a wide range of therapeutical properties. Both roots and leaves are used topically and orally in the form of a crude extract or infusion and the roots have been identified as the most active part of the plant [2]. It is considered an antispasmodic, diuretic, menstrual promoter, stimulant, and sweat promoter. Herbalists and natural health practitioners use it for edema, arthritis, malaria, rheumatism, poor memory, as a topical analgesic and anti-inflammatory for skin afflictions. A fraction of *Petiveria alliacea* leaves and stems were also discovered to induce *in vitro* cell death and *in vivo* tumor regression in a murine breast cancer model [3]. Their results validate in part, the traditional use of *Petiveria alliacea* in breast cancer treatment, revealing a new way of envisioning *Petiveria alliacea* biological activity. The fraction effect on the glycolytic pathway enzymes contributes to and explained the anti-proliferative and antitumor activities.

When crushed, and in some areas of tropical America it serves as an insect and bat repellent, and as an acaridice [5,6]. In Latin America and the West Indies, it is commonly used by "medicine men" in ritual ceremonies. In Brazil, Cuba, and tropical Africa it is important in Yoruba magical rituals [1].

The aqueous extract of *Petiveria alliacea* was reported by Christie and Levy [6] to have demonstrated a hyperglycaemic effect in normoglycaemic rats and showed no hypoglycaemic activity in diabetic rats. According to them, the hexane extract caused no hypoglycaemic action in normal rats and failed to sustain an initial hypoglycaemic action in diabetic rats.

Despite its enormous usefulness, it is surprising to note that it was reported that this plant often cause dermatitis in humans and taints the milk and meat of animals when they graze on it and may also induce abortion [7,8]. When this species is fed to animals on a regular basis it may cause adverse reactions. The ability of the plant to accumulate nitrates may also cause nitrate poisoning in cattle [9]. *Petiveria alliacea* is a common weed in coffee, maize, and apple plantations as well as in pasturelands and natural forests [8,4,10-11].

Despite the fact that a lot of researches have been done on *Petiveria alliacea* in the past fifty years, it was observed recently at a University Teaching Hospital in Nigeria that the plant is still being used in treating other ailments that are yet to be documented. This necessitated the present research to verify other possible active compounds in the plant that have not been recorded earlier on.

Materials and Methods

Collection and Preparation

Petiveria alliacea materials (about 2kg of leaves and root) were collected from 'OmoOgbe' farm at Oluponna area of Osun State in January, 2016. They were then taken straight to the laboratory to be washed, separated and stored in the freezer temperature (about -20^{o}C) until the time of use.

Soxhlet extraction method was used for the extraction of crude materials from the *Petiveria alliacea* plant (leaves and root) using n-hexane, chloroform, methanol and water as solvents after which they were concentrated.

The surface of the wet plant materials from the freezer were reduced to smaller particles for extraction. Tin layer chromatography was employed to obtain pure fractions which were used for bio assays.

Column chromatography was employed for the purification and separation of the crude extracts into different components using Oxford Laboratory silica gel (100-200 mesh) as the solid adsorbent usually referred to as the stationary phase, while different ratios of the solvents discovered from the TLC were used as eluents to achieve good separations.

Antibacterial Activity

Antibacterial activity of both chloroform and methanolic extracts and fractions were determined using filter paper disc method. The pure culture of each bacterium was inoculated in peptone water for 24 hours after which it was

inoculated into Mueller Hinton agar. The sterile discs were soaked in the test extracts and allowed to dry for about 15 minutes. Each disc was placed into the MHA bacteria seeded plate and left at room temperature for 5 hours to enhance the diffusion of extracts into the culture medium. The petri dishes were incubated for 24 hours at 37^{o}C. The zones of inhibition were then observed and measured with the aid of meter rule. The experiment was carried out in duplicate. Presence of zones of inhibition around each of the paper discs signified the presence of anti-bacterial action.

Antifungal Activity

This was also carried out using filter paper disc method. The sterile discs were soaked with the test extracts and allowed to dry for 15 minutes. Each disc was introduced into the PDA fungi seeded plate and left at room temperature for 5 hours to enhance diffusion of extract into the culture medium. The petri dishes were incubated for 3 days at 28^{o}C. The zones of inhibition were then observed and measured with in millimeter (mm).

Results and Discussion

Antimicrobial Activity

The antibacterial and antifungal activities of the crude extracts and chromatographic fractions were carried out. The results are presented in Tables 1-8 as shown below. Note that for each table; A-F represents different fractions obtained from column chromatography of the crude extracts.

Table 1: Antibacterial activities of methanolic extract and fractions of *Petiveria* alliacea roots

Extracts	**Microorganism/Zone of Inhibition (mm)**			
	E-Coli	***Bacillus subtilis***	***Micrococcus sp***	***Staphylococcus aureus***
Control	-	-	-	-
Crude extracts	8.0±0.0	11.0±1.4	7.5±0.7	9.0±0.0
A	7.5±0.7	3.5±5.0	3.5±5.0	3.5±5.0
B	9.0±1.4	3.5±5.0	-	-
C	7.0±0.0	4.0±5.7	3.5±5.0	-
D	7.0±0.0	-	4.0±5.7	3.5±5.0
E	7.5±0.7	-	3.5±5.0	-
F	7.0±0.0	-	7.0±0.0	8.0±0.0

Methanol was used as the control being the solvent of extraction. A was extract obtained from 100% chloroform B was a mixture of 80% chloroform and 20% methanol. C was a mix of 60% chloroform and 40% methanol while D was a mix of 40% chloroform and 60% methanol. E and F were obtained from 100% methanol.

Table 2: Antibacterial activities of methanolic extract and fractions of *Petiveria alliacea* leaves

Extracts	**Microorganisms/Zone of inhibition (mm)**			
	E-Coli	***Bacillus subtilis***	***Micrococcus sp***	***Staphylococcus aureus***
Control	-	-	-	-
Crude extract	9.0±1.4	8.0±0.0	7.0±0.0	4.0±5.7
A	9.0±1.4	-	3.5 ±5.0	-
B	7.0±0.0	-	-	-
C	8.0±1.4	4.0±5.7	-	-
D	4.5 ±6.4	-	-	-
E	5.5 ±7.8	-	-	-
F	5.0±7.1	4.0±5.7	-	-

Methanol was also used as the control. A was extract obtained from chloroformand methanol the ratio 3:2 respectively while B was a mixture of chloroform and methanol in the same ratio. C was a mix of 40% chloroform and 60% methanol while D was a mix of 10% chloroform and 90% methanol. E and F were obtained from 100% methanol.

Table 3: Antibacterial activity of chloroform extract and fractions of *Petiveria alliacea* leaves

Extracts	Microorganisms/Zone of inhibition (mm)			
	E-Coli	***Bacillus subtilis***	***Micrococcus sp***	***Staphylococcus aureus***
Control	-	-	-	-
Crude extract	10.0±4.2	-	7.5±0.7	-
A	4.0±5.7	9.5 ±0.7	7.0±0.0	-
B	6.0±8.5	8.5 ±0.7	7.0±0.0	5.0±7.1
C	9.0±1.4	7.5±0.7	7.0±0.0	-
D	9.0±1.4	8.5 ±0.7	7.0±0.0	6.0±8.5
E	-	4.0±5.7	7.0±0.0	-

Methanol was also used as the control. A was extract obtained from petroleum ether and ethyl acetate the ratio 9:1 respectively while B was a mixture of petroleum ether and ethyl acetate the ratio 3:2 respectively. C was a mix of petroleum ether and ethyl acetate the ratio 1:1 while D was a mix of 35% petroleum ether and 65% ethyl acetate. Ewas a mix of petroleum ether and ethyl acetate the ratio 1:4 respectively.

Table 4: Antibacterial activity of chloroform extract and fractions of *Petiveria alliacea* roots

Extracts	Microorganisms/Zone of inhibition (mm)			
	E-Coli	***Bacillus subtilis***	***Micrococcus sp***	***Staphylococcus aureus***
Control	-	-	-	-
Crude extract	9.0±2.8	9.0±1.4	-	8.0±0.0

Table 5: Antifungal activities of methanolic extract and fractions of *Petiveria alliacea* roots

Extracts	Microorganisms/Zone of inhibition (mm)				
	Penicillium sp	***A/flavus***	***Collectotrichum sp***	***Trichoderma sp***	***Rhizopus sp***
Control	-	-	-	-	-
Crude extracts	3.5 ±5.0	-	-	-	-
A	3.5 ±5.0	-	-	-	-
B	4.0±5.7	-	5.0±7.1	-	9.5±0.7
C	-	-	-	-	-
D	-	-	4.0±5.7	-	-
E	3.5 ±5.0	-	-	-	5.0±7.1
F	-	-	3.5±5.0	-	5.5 ±7.8

Chloroform was used as the control. A was extract obtained from 100% chloroform while B was a mix of chloroform and methanol in the ratio 4:1 respectively. C was a mixture of chloroform and methanol in the ratio 3:2while D was the same mixture in the ratio 2:3 respectively. E and F were 100% methanol.

Table 6: Antifungal activities of methanolic extract and fractions of *Petiveria alliacea* leaves

Extracts	Microorganisms/Zone of inhibition (mm)				
	Penicillium sp	***A/flavus***	***Collectotrichum sp***	***Trichoderma sp***	***Rhizopus sp***
Control	-	-	-	-	-
Crude extract	4.5 ±6.4	-	8.5 ±0.7	-	-
A	-	-	-	-	-
B	5.0 ±7.1	-	8.0 ±1.4	-	-
C	5.0 ±7.1	-	-	-	-
D	-	6.5 ±9.2	4.0 ±5.7	-	-
E	4.0 ±5.7	-	5.5 ±7.8	-	-
F	-	11.0 ±4.2	-	-	-

Methanol was used as the control. A was a mix of chloroform and methanol in the ratio 3:2 while B was a mix of the same mixture but in the ratio 1:1. C was chloroform and methanol in the ratio 2:3 and D was same mixture of chloroform and methanol in the ratio 1:9 respectively. E and F were 100% methanol extracts.

Table 7: Antifungal Activity of chloroform extract and fractions of *Petiveria alliacea* leaves

Extracts	Microorganisms/Zone of inhibition (mm)				
	Penicillium sp	***A/flavus***	***Collectotrichum sp***	***Trichoderma sp***	***Rhizopus sp***
Control	-	-	-	-	-
Crude extracts	9.0 ±0.0	4.0 ±5.7	8.5 ±0.7	11.0 ±0.0	6.0 ±8.5
A	-	9.0 ±0.0	6.0 ±8.5	-	10. 0 ±0.0
B	-	-	5.0 ±7.1	5.0 ±7.1	6.0 ±8.5
C	9.0 ±2.7	4.0 ±5.7	9.5 ±0.7	5.0 ±7.1	-
D	5.0 ±5.7	5.0 ±7.1	-	4.5 ±6.4	4.5 ±6.4
E	3.5 ±5.0	4.0 ±5.7	-	9.5 ±0.7	-

The Control here was also methanol. A, B, C, D and E were mixtures of petroleum ether and ethyl acetate in the ration 9:1, 3:2, 1:1, 1:2 and 1:4 respectively.

Table 8: Antifungal Activity of Chloroform extracts and fractions of *Petiveria alliacea* roots

Extracts	Microorganism/Zone of inhibition (mm)				
	Penicillium sp	***A/flavus***	***Collectotrichum sp***	***Trichoderma sp***	***Rhizopus sp***
Control	-	-	-	-	-
Crude extracts	11.0± 1.4	5.0± 7.1	6.5 ± 9.2	3.5 ± 5.0	-

The antimicrobial test was carried out in two phases: antibacterial and antifungal. The antibacterial activities of the methanolic extracts of the root as shown in Table I indicated the strong antibacterial activity of the root against the following bacteria: *Staphylococcus aureus, Escherichia coli, Micrococcus sp and Bacillus subtilis*. Apart from the crude which understandably has the strongest activity against all the bacteria, fraction 'A' was the only one with broad spectrum activity. A close look at the table revealed that all the fractions except 'B' had activity on *Micrococcus sp* while all of them had activity with *Escherichia coli*. These results showed that the methanolic extract of the root can be depended upon for antibacterial activity.

Table II revealed the activities of methanolic extract of the leaves against the four bacteria. The whole fractions acted strongly on *E. coli*, few fractions (C & F) on *Bacillus subtilis*, a fraction (A) on *Micrococcus sp* while none on *Staphylococcus aureus*.

Also, the antibacterial activities of the chloroform extracts as shown in tables IV & V revealed that chloroform extracts of leaves have more efficacy than its root extracts. Almost all the chloroform fractions acted on all the bacteria as against the root extracts that acted only on three but was not active against micrococcus.

These revealed clearly that the methanolic extract of the root has more effect on bacteria than the methanolic extracts of the leaves. While the chloroform extracts of leaves are more effective on the bacteria than the root extracts.

Antifungal

Tables 5-8 revealed the antifungal activities of both the methanolic and chloroform extracts of both the leaves and roots. The tested fungi include: *Penicillium sp, Aspergillus flavus, Collectotrichum sp, Trichoderma sp and Rhizopus sp.* Table VI showed the activities of the methanolic extract and fractions of roots against some fungi. The fractions were active against three fungi: *Penicillium sp, Collectotrichum sp* and *Rhizopus sp*. The highest activity was from fraction 'B' against *rhizopus sp*, followed by 'F' against the same *rhizopus*. They were completely inactive against both *A/flavus* and *Trichoderma sp*.

Table 7 revealed the activities of the methanolic extract and fractions of the leaves against the same set of fungi with slightly different results. Fractions 'B, C, E' were active against *Penicillium sp*, while 'D & F' showed actions on A/flavus and 'B, D & E' against *Collectrichum sp*. It was surprisingly observed that fraction 'A' was completely inactive against all the fungi and none of the fractions were active against both *Trichoderma sp* and *Rhizopus sp*.

The chloroform extracts as shown by tables 8 & 9 revealed a lot of activities from the leaves with fraction 'A' having the highest activities for *A/flavus, Collectotricum sp* and *Rhizopus sp*. While the leaves revealed activities across the five fungi, the root extract was only active on four but inactive with *Rhizopus sp*.

From the antifungal activities, though both the root and the leaves are efficacious, it can be concluded that the leaves are more effective for fungi treatments than the root.

Table 9: Results of the phytochemical analysis of extracts of *Petiveria alliacea*

Extracts	Alkaloids	Flavonoids	Tannins	Saponins	Terpenoids	Steroids
WR	_	+	+	_	+	+
MR	_	+	+	+	+	+
CR	+	_	_	_	+	+
CL	_	_	_	_	+	+
ML	_	+	+	_	_	+

Keys:
+ = present , - = absent
WR = water extract of root
MR = methanolic extract of root
CR = chloroform extract of root
CL = chloroform extract of leaves
ML = methanolic extract of leaves

Phytochemicals Analysis

Table X shows the result of the phytochemical analysis of different extracts of the plant of *Petiveria alliacea*. From the results, saponins are absent in all the extracts except MR, while alkaloids are absent in all but CR. Both flavonoids and tannins are present in WR, MR and ML but absent in CR and CL. Terpenoids are present in all but ML while steroids are present in all. The presence of these secondary metabolites (particularly in the root extracts from the more polar solvents) explains the industrial and medicinal importance of the plant.

Conclusion and Recommendation

The medicinal properties of *P*etiveria *alliacea* have been further expounded from this research work. Its strong anti-microbial activities have been further stretched over different bacteria and fungi as well as phytochemical properties which confirms its medicinal properties. With the discovery of different white crystals from some of the extracts (which will be reported in another publication as soon as they are identified), it shows that more research work are still required on this plant in order to fully maximize its potentials. All these are indication that there are still many compounds that are yet to be discovered in this plant. By the time analysis such as NMR and MS are carried out on all of these fractions, a good conclusion can then be arrived at.

It is therefore recommended that more attention be given to the plant in order to get all that nature has deposited in it for the use of humanity. Of course, in the subsequent work, the identities and structures of these fractions will be confirmed using mass spectroscopy (MS) and nuclear magnetic resonance (NMR).

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