Centrepoint Journal (Science Edition) 2141-3819/2017 $5.00 + 0.00
Volume 24, No.2, pages 141-174
http//www.unilorin.edu.ng/centrepoin
CPJ 2018022/24210

Extraction, Physicochemical, Phytochemical, Biochemical, GC-MS Constituents and Environmental Effects of *Petiveria alliacea* Leaves

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Abstract

The study was carried out to determine the phytochemical compositions, physicochemical parameters, biochemical and Gas Chromatography-Mass Spectroscopy GC-MS analysis of *Petiveria alliacea* leave extracts collected at three different times of the day and the results compared. The extracts were prepared using successive extraction with both non-polar and polar solvents. The physicochemical analysis revealed no significant variation in the three samples. It showed that the extracts are green in colour and acidic with a total ash content of 18.95 %. The phytochemical analysis revealed that flavonoids, terpenoids, alkaloids, saponins, steroids, glycosides, tannins were present in the plant leaves but the composition varies in relation to the time of collection of the samples. GC-MS analysis revealed major constituents such as; 9,12,15-octadecatrienoic acid; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Squalene and n-Hexadecanoic

acid in the n-Hexane extracts of the morning and afternoon samples and major constituents of Phytol, α-linolenic acid, Pinane, Palmitic acid and 5-nonadecen-1-ol in the evening sample. In the ethanol extract, n-Hexadecanoic acid, phytol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and 9,12-Octadecadienoyl chloride were found in the morning sample; campesterol, 9,12,15-octadecatrienoic acid and ethyl ester in the afternoon sample while Linolenic acid, Nonadecyl cyclohexane and 9,12,15-octadecatrienoate in the evening samples. It is thus concluded that *P.allliacea* leaves contain many bioactive components and that collection times and the extraction medium have significant effects on the chemical compositions of the leaves. These compounds possess many biological properties; however, these results confirmed the influence of the time of collection of the leaves on the composition which affect the biological activity and pharmacological applications of the plant. The variation in the constituents in the samples could be attributed to sunlight effect, since all other conditions are the same. Also leaf extracts of *P.alliacea* collected at three different times of the day showed varying and different toxicological effects when administered to Wistar rats.

Keywords: Phytochemicals, *Petiveria alliacea*, GC-MS, Physicochemical, Biochemical parameters

Introduction

Plant is man's friend in survival, giving him food, fuel and medicine from the days beyond dawn of civilization. Medicinal plants are those plant parts such as (stems, roots, leaves' extracts etc.) that are used in treating and preventing specific ailments and diseases that affect human beings (Akinmoladun *et al*., 2007). Medicinal plants contain both organic and inorganic constituents, and are found to be rich in one or more individual elements, thereby, providing a possible link to the therapeutic properties of the medicines (Singh and Garg, 1997). Many active compounds include; alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids, which are deposited in their specific parts, such as leaves, flowers, barks, seeds, stems, fruits, and roots. The beneficial medicinal effects of these plant materials typically result from the combination of some of these secondary products (Tonthubthimthong *et al*., 2001).

Petiveria alliacea (P. alliacea) is a perennial shrub in the pokeweed family (*Phytolaccaceae*), which grows wildly in the South and Central American tropics, Africa and the Caribbean (Garcia-gonzalez, 2006). *P. alliacea* is known by a wide number of common names including: anamu in the Dominican Republic, Puerto Rico in Brazil (where it is also known as tipi), mucura in Peru, guine in many other parts of Latin America, mapurite (pronounced Ma-po-reete) in Trinidad, in Mexico as gully root and in Jamaica as guinea hen weed (Mendes,1986). It is sometimes called "garlic weed" as the stems and roots have a strong garlic odour, which taints the milk and meat of animals that graze on it. *Petiveria alliacea* is described as a tall straight perennial herb, slightly branched and is 0.5 to 1 m tall, with alternate leaves in elliptic form, 6 to 19 cm long and up to 5 cm wide. Its flowers are small and white, bisexual, zygomorphic, slightly imbricate to rather remote; sepals are white or greenish to pinkish, linear-lanceolate to linear-oblong, 3.5-6 mm long with superior ovary; fruit is a cuneiform berry, narrowly oblong achenes, 6-8 mm long with four hooks turned downwards (Illnait, 2007; Alegre and Clavo, 2007); Seeds are solitary, erect and linear.

P. alliacea plants have been used for pain relief, as an anti-influenza, anti-inflammatory, anti-tumor, anti-bacterial, anti-fungal, anti-hyperlipidemia, anti-diabetic, anti-venereal, sedative, anti-helminthic, emmenagogue, anesthetic, and anti-cancer drug (Schmelzer and Gurib-Fakim, 2008; TPD, 2011; Hernandes *et al*., 2014). It is used in folk medicine to enhance memory and in the treatment of common cold, flu, other viral or bacterial infections (Kim *et al*., 2006), and it has depurative properties (De Lima *et al*., 1991).

Due to the various reported biological effects of this plant, there arises a need to screen the plant for bioactive compounds. Also, secondary metabolism is known for showing response patterns related to environmental stimuli, and it is thought that environmental temperature might play a major role in the composition and metabolic activity of the plant (Blank *et al.*, 2005). This study aimed at carrying out phytochemical, physicochemical, and GC-MS analysis on the leaves' extracts of *P. alliacea* and assessing the effects of collection time and extraction medium on the chemical constituents. Similarly, despite the therapeutic uses of medicinal plants and medicinal plant products, the toxicological effects and effective dosage of most of these plants has not been

investigated to the best of our knowledge. Therefore, this research also determined the toxic and safety effects of n-hexane and ethanol extracts of *P. alliacea* leaves, collected at different times of the day (morning, afternoon and evening).

Materials and Methods

Chemicals and reagents

The assay kits for urea, creatinine, albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were products of RANDOX Laboratory Limited, Cumlin, UK. All other reagents used were of analytical grade and supplied by Sigma-Aldrich Inc., St. Louis, USA.

Distilled water, double-distilled ethanol and n-hexane, Ferric chloride, glacial acetic acid, chloroform, hydrochloric acid, concentrated sulphuric acid and Dragendorff reagent.

Plant Sample Collection and Preparation to Powder Form

The leaves of *P. alliacea* were collected at a flower garden in Ilorin, Kwara state, Nigeria at three different times (6:00 am, 1:00 pm and 6:00 pm) of the day. Identification was done at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Kwara State and voucher specimen number UILH/001/1224 was deposited. The leaves were thoroughly washed with water, air dried until constant weights were obtained (163.28, 231.33 and 166.94 g) for morning, afternoon and evening samples respectively. The dried leaves were then pulverized using mortar and pestle and stored in an air tight, sealed plastic container at room temperature till the time of extraction.

Preparation of Plant Extracts

All solvents used in the extraction process were double-distilled using a distillation apparatus. The *P.alliacea* leaves' extracts were prepared using cold successive extraction at room temperature. The pulverized samples were soaked in extract jars with double-distilled n-hexane, left for three days (72 hours) with frequent agitation and then decanted. Double-distilled ethanol solvent was successively added to the samples in the jars and also left for another three days (72 hours) with frequent agitation

before being decanted. The extracts were then filtered using a Whatmann No.1 filter paper (Sringfield, Maidstone, Kent and England) and concentrated using a rotary evaporator (Buchi Rotavapor R110 Laboratories-Technik Ag. CH-9230 FLAWIL/SCHWE12, made in Switzerland) at 40 ºC and kept at a temperature of 4°C until further analysis.

Experimental Animals

Twenty-one (21) healthy adult male Wistar rats weighing 165.5 g were obtained from the Animal House of Biochemistry Department, University of Ilorin, Ilorin, Nigeria. The animals were housed in well-ventilated plastic cages with sawdust as beddings and fed on standard rodent feed and water *ad libitum*. The animals were acclimatized for two weeks before treatment. Appropriate ethical clearance had been obtained.

Animal Grouping

All rats were maintained under standard laboratory conditions (12-h light/dark cycle, 25 ± 2°C). The animals were then randomly distributed into seven groups, each with three rats. Group A is the positive control and Groups B to G are the test groups. Table 1 shows a list of the groups.

Table 1: Animal Grouping

Test Groups	**Extracts administered daily**
A	Control: distilled water
B	*Petiveria* ethanol morning
C	*Petiveria* n-hexane morning
D	*Petiveria* ethanol afternoon
E	*Petiveria* n-hexane afternoon
F	*Petiveria* ethanol evening
G	*Petiveria* n-hexane evening

Collection of Blood Samples and Preparation of Serum

After two weeks of administration of the extracts, the animals were sacrificed after inhalational anaesthesia with di-ethyl ether. This was followed by blood collection by cardiac puncture into sterile plain bottles for analysis. Blood samples were allowed to stand for 30 minutes, after which it was centrifuged at 1000 g for 10 minutes. The collected serum after centrifugation were appropriately labeled and stored in a freezer at -4 ^{o}C until required for analysis.

Isolation and Homogenization of Tissues

During the sacrifice, the rats were dissected and a known weight of selected tissue were isolated (liver, heart and kidney). The isolated tissues were cleaned with cotton wool to remove blood stains, weighed and immediately stored in ice cold 0.25 M sucrose solution. The isolated organs were subjected to homogenization in ice-cold 0.25 M sucrose solution (1:5 w/v). The homogenates were stored in the freezer (-4 ^{o}C) before used for analysis.

Preliminary Phytochemical Screening of Extracts

Qualitative tests for the presence of active compounds such as steroids, glycosides, saponins, alkaloids, flavonoids, tannins and phenols were carried out on the n-hexane and ethanol extracts, using standard procedures (Harborne, 1984, Trease and Evans, 1989, Sofowora, 1996).

Physicochemcal Analysis

The pH, specific gravity, colour, moisture and ash content of *P. alliacea* leaves' extracts were determined. The pH of each extract solution was measured using a pH meter (Combo meter, New Zealand) and the colour was determined by measuring the absorbance of the extract solution on a Beckman Coulter DU 730 UV/VIS spectrophotometer. The moisture and ash contents were determined using standard methods (AOAC, 1980).

GC-MS Analysis

Three gram (3 g) each of the *P. alliacea* leave extracts were re-dissolved in 5 ml n-hexane and ethanol in two different sample tubes before they were subjected to GC-MS analysis for characterization.

Some Biological activities of reported Bioactives in *Petiveria*

The biological activities of some of the compounds identified as published by a researcher are as summarized in Table 2.

Table 2: Biological activities of reported Bioactives

S/No.	Name of compounds	Compound's nature	Biological Activity
1	Hexadecanoic acid, methyl ester	Organic acid	Antimicrobial
2	n-Hexadecanoic acid	Palmitic acid	Antimicrobial, Lubricant, Antioxidant, Hemolytic Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic, Flavour.
3	Squalene	Triterpene	Antibacterial, anticancer, Antioxidant, Pesticide, Antitumor, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Adjuvant
4	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol	Antimcrobial, Antituberculosis, insecticidal, anti-inflammatory, antioxidant
5	Phytol	Diterpene	Antimicrobial, Anticancer, Cancer preventive, Diuretic Anti-inflammatory,
6	Hexadecanoic acid, ethyl ester	Ester compound	Antioxidant, Flavor, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Hemolytic, 5-Alpha reductase inhibitor
7	Hexadecanoic acid, methyl ester	Fatty acid ester	Antioxidant, Flavor, Hypocholesterolemic Pesticide, 5-Alpha reductase inhibitor
8	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Fatty acid ester compound	Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge Antihistaminic, Antiarthritic, Anticoronary, Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic,
9	Stigmasterol		Anti-inflammatory, inhibit tumor promotion, anti-HIV reverse transcriptase, anti-inflammatory
10	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	Phytol	Antimicrobial Anticancer Anti-inflammatory Diuretic

Source: Duke's Phytochemical and Ethnobotanical Databases (2016)

Roles of identified fatty acids in soap making

The fatty acids; stearic acid, palmitic acid, myristic acid, lauric acid and oleic acid contribute to lathering and washing properties of the soaps. These are characteristically different from soaps made from divalent metals such as calcium, magnesium, iron or aluminum which are not water soluble (Shoge, 2011).

Cosmetic effects

The presence of phytochemicals such as antioxidants, vitamins, proteins, tannins, terpenoids and other bioactive ingredients help rejuvenate, freshen and protect the hair and skin from various skin and hair conditions such as psoriasis, eczema, skin dryness, skin cancers, sun burn, skin dryness, boil, solar keratosis, dermatitis, impetigo, candidiasis, athlete's foot, chicken pox, carbuncles, staph infections, cyst, abscess, cracking, dandruff, flaking and others.

Increased attention has been given to the use of natural antioxidants for prevention of diseases caused by oxidative damage in human body and/or by lipid peroxidation in food (Teow *et al.,* 2007).

Biochemical Assays

Total Protein Determination

The protein content of the serum and homogenates was determined using the Biuret method as previously described by Sulaiman and Adeyemi, 2010.

Determination of other Biochemical Parameters

The concentrations of urea, creatinine and albumin (ALB), as well as the enzyme activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were done according to the methods described in the RANDOX Laboratory assay kits.

Statistical Analysis

All data were expressed as the mean of 3 replicates ± standard error of mean (S.E.M). Statistical evaluation of data was performed by Graph pad prism version 5.02 using one way analysis of variance (ANOVA). Post-Hoc test analysis was done using the Tukey's Multiple Comparison Test. Values were considered statistically significant at $P < 0.05$.

Results and Discussion

Results of the Phytochemical Analysis

The results of the phytochemical analysis carried out on the extracts of *Petiveria alliacea* leaves collected in the morning (6:00 am.), afternoon (1:00 pm) and evening (6:00 pm) are as summarized in table 4:

Table 4: Phytochemical composition of *Petiveria alliacea* leaves collected at different times of the day

Phytochemicals	Morning (6:00 am)		Afternoon (1:00 pm)		Evening (6:00 pm)	
	n-Hexane	Ethanol	n-Hexane	Ethanol	n-Hexane	Ethanol
Alkaloids	+	+	-	-	+	-
Tannins	+	+	-	+	-	+
Flavonoids	+	+	+	-	-	+
Glycosides	+	+	+	+	-	+
Steroids	+	-	+	+	-	-
Saponins	+	+	-	+	+	+
Phenols	-	-	-	+	-	-
Terpenoids	+	+	+	-	+	-

Key: + present: - absent

The phytochemical screening of the n-hexane and ethanol extracts of the leaf of *Petiveria alliacea* revealed that tannins, glycosides, terpenoids, flavonoids and saponins are present in almost all the extracts, which shows that the plant is rich in these metabolites.

Flavonoids, tannins and phenols are phenolic compounds; they are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found to be present in the extracts, they may be responsible for the potent antioxidant capacity of *Petiveria* leaf. Previous studies have also reported that phenolic compounds possess other biological activities such as anti-inflammatory,

anti-ulcer, anti-spasmodic and anti-diarrheal (Galm and Bashen, 2007; Djeridane *et al*., 2006). Alkaloids, according to literature, possess anti-inflammatory, anti-asthmatic, and anti-anaphylatic properties with consequences of altering immunological status in vivo study (Johnson *et al*., 2012). Saponins are special class of glycosides that have been shown to possess anti-fungal activity, hypolipidermic and anti-cancer activity (Sarker and Nahar, 2007). Steroids have also been reported to possess antibacterial, anti-inflammatory, anti-ulcerative and anti-tumor actions (Kim *et al.,* 2006).

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Saponins are special class of glycosides that have been shown to possess anti-fungal activity, hypolipidermic and anti-cancer activity (Sarker and Nahar, 2007). Steroids have also been reported to possess antibacterial, anti-inflammatory, anti-ulcerative and anti-tumor actions (Kim *et al.,* 2006). However, there is variation in the phytochemicals obtained from the leaves in relation to the collection times. For instance, alkaloids are present in both n-hexane extracts of leaves collected in the morning and in the evening but absent in those collected in the afternoon (Table 3). Also, Phenol is absent in all the extracts except in the ethanol extract of leaves harvested in the afternoon while steroids are present in both morning and afternoon samples but not in the evening sample. These observations are indication that the time of collection depends on the ailment to be treated that is the metabolite of interest.

Physicochemical Analysis of Extracts

The results are as summarized in Tables 4a and 4b:

Table 4a: Results of physical parameters of *Petiveria alliacea* leaves collected at different times of the day

Physicochemical Parameters	Morning		Afternoon		Evening	
	n-Hexane	Ethanol	n-Hexane	Ethanol	n-Hexane	Ethanol
pH	4.58	5.836	6.955	6.342	6.129	5.777
Color	Green	Green	Green	Green	Green	Green
Specific gravity	0.8355	0.8148	0.633	0.801	0.7147	0.8299

Table 4b: Result of physicochemical parameters of *Petiveria alliacea* leaves collected at different times of the day

Physicochemical Parameter	Morning (6:00 a.m.)	Afternoon (1:00 p.m.)	Evening (6;00 p.m.)
Moisture content (%)	79.00	80.94	80.74
Total ash content (%)	18.59	18.59	18.59

As shown in table 4b, there was no significance variation in the physicochemical parameters in relation to the collection time. A total ash content of 18.59 % indicates that the amount of organic matters and other minerals in the leaves of *P. alliacea* is slightly high. It also showed that the extract is acidic (4.58) and green in colour (Table 4a).

Results of GC-MS Analysis

As revealed by GC-MS analysis, in n-hexane extracts 14 compounds were present in the morning, 17 compounds in the afternoon and 10 compounds in the evening (Table 5). However, in ethanol extracts, 13 compounds were present in the morning, 11 in the afternoon and 17 compounds in the evening extracts (Table 6).

Table 5: GC-MS results of n-hexane extracts of morning, afternoon and evening samples

S/No.	Morning	%	Afternoon	%	Evening	%
1	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	16.80	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	17.22	Phytene 2	1.32
2	2(4H)-Benzofuranone,5,6,7,7a tetrahydro-4,4,7a trimethyl-, (R)-	1.49	2(4H)-Benzofuranone, 5, 6,7,7a-tetrahydro-4,4,7a-trimethyl	1.20	Pinane	11.21
3	2-Hexadecene,3,7,11,15-tetramethyl-[R-[R*,R*-(E)]]-	1.76	2-Hexadecen-3,7,11,15-Tetramethyl	1.51	5-nonadecen-1-ol	6.08
4	N-Hexadecanoic acid	11.07	N-Hexadecanoic acid	9.38	Palmitic acid	9.86
5	Hexadecanoic acid, ethyl ester	1.99	Hexadecanoic acid, ethyl ester	5.53	Squalene	2.10
6	Phytol	9.22	Phytol	8.02	Phytol	43.97
7	9,12,15-Octadecatrienoic acid,	32.51	9,12,15-octadecatrienoic acid	22.06	Ethyl palmitate	1.42
8	Octadecanoic acid	2.19	Ethyl-9,12,15-octadecatrienoate	9.23	Alpha linolenic acid	15.13
9	Ethyl iso-allocholate	4.04	Ethyl iso-allocholate	1.79	Linolenic acid, ethyl ester	5.73
10	Cholestan-3-one, cyclic 1, 2-ethanediyl acetal	1.02	4,8,12,16-	0.69	Palmitin	3.19

	(5β)		Tetramethylheptadecan-4-olide			
11	7, 8- Epoxylanostan-11-ol, 3-acetoxy-	2.31	Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediylester	1.62	-	
12	Diisooctyl phthalate	2.11	Diisooctylphthalate	1.76	-	
13	Stigmasterol	1.42	1-heptatriacotanol	2.67	-	
14	Squalene	14.81	Squalene	12.57	-	
15	-		Octadecanoic acid,ethyl ester	1.30	-	
16	-		Docosanoic acid-1,2,3-propanetriylester	1.13	-	
17	-		Oleic acid, eicosyl ester	2.33	-	

NOTE:- sign – denote no compound detected.

From the GC-MS data, the variation in the compositions is morning < afternoon > evening for n-hexane sample extracts but significantly high in the afternoon while the order is morning > afternoon < evening for ethanol sample extracts but significantly high in the evening sample. Many compounds present in both the n-hexane extracts of the morning and afternoon samples; include 2(4H)-Benzofuranone,5,6,7, 7a tetrahydro-4,4, 7a trimethyl-, (R)- ; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; 2-Hexadecene,3,7,11,15-tetramethyl-[R-[R*,R*-(E)]]-; N-Hexadecanoic acid; Hexadecanoic acid, ethyl ester; Phytol; Ethyl iso-allocholate; Squalene and 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- in varying concentrations (Table 5).

In the ethanol extract, also present in the morning and afternoon samples are; Phenol,2,4-bis(1,1-dimethylethyl; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Hexadecanoic acid, methyl ester; Hexadecanoic acid, ethyl ester; 9,12,15-octadecatrienoic acid, ethyl ester; Stigmasterol; and Campesterol, n-Hexadecanoic acid, and Phytol are also present in the evening sample (Table 6).

Table 6: GC-MS result of Ethanol extracts of morning, afternoon and evening samples

S/No.	Morning	%	Afternoon	%	Evening	%
1	1-(2-Hydroxyethyl)-1,2,4-triazole	2.47	Cholestan-3-ol,2-methylene-	5.57	Nonadecyl cyclohexane	9.15
2	Phenol, 2,4-bis(1,1-dimethyl)	2.06	Phenol,2,4-bis(1,1-dimethylethyl	2.88	2(E)-hexenoicacid (4s)-amino 5-methyl	5.79
3	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	8.46	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	4.12	Ethylene diammine	1.51
4	n-Hexadecanoic acid	10.37	n-Hexadecanoic acid	7.48	n-Hexadecanoic acid	13.05
5	Hexadecanoic acid, methyl ester	2.35	Hexadecanoic acid, methyl ester	1.79	2,4-di-tert butyle phenol	1.90
6	Hexadecanoic acid, ethyl ester	1.49	Hexadecanoic acid,ethyl ester (3.19 %)	3.19	2,6,6-trimethyl-bicyclo(3,1,1) heptane	1.12
7	Phytol	27.15	Phytol (36.68 %)	36.68	Phytol	24.72
8	9,12-Octadecadienoyl chloride, (Z,Z)-	10.66	Cholest-5-en-3-ol,24-pyrolidene (3.12 %)	3.12	4-hydroxy-trimethyl pyroline	5.82
9	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	17.08	9,12,15-octadecatrienoic acid,ethyl ester (20.15 %)	20.15	9,12,15-octadecatrienoate	7.57
10	Stigmasterol	2.32	Stigmasterol	1.16	Methyl palmitate	2.11
11	Campesterol	3.94	Campesterol	13.87	Linolenic acid	14.70
12	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl	3.15	-		Methyl ricinoleate	3.31

	ester					
13	1-Heptatriacotanol	2.73	-		Cyclopropane (3-chloropropyl) methylene	1.45
14	-		-		Fumaric acid,hexadecyl propagyl ester	1.38
15	-		-		Octadecanedioic acid	4.21
16	-		-		3-methoxy,-3,4-dimethyl-1-heptyne	1.30
17	-		-		2-oxa-spiro(4.50 dec-8-ene-1,7,-dione,4,6-dihydroxy-3,10-dimethyl	0.86

Key: - absent

The main constituents in the n-hexane extract of the leaves plucked in the morning and afternoon include: 9,12,15-octadecatrienoic acid; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Squalene and n-Hexadecanoic acid while Phytol, α-linolenic acid, Pinane, Palmitic acid and 5-nonadecen-1-ol are the major constituents in the evening extract (Table 5). Furthermore, main constituents present in the ethanol extract of the morning, afternoon and evening samples include; n-Hexadecanoic acid, phytol, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and 9,12-Octadecadienoyl chloride, (Z,Z)- in the morning sample, campesterol, 9,12,15-octadecatrienoic acid, and ethyl ester in the afternoon sample and Linolenic acid, Nonadecyl cyclohexane and 9,12,15-octadecatrienoate in the evening sample (Table 6).

Result of Biological Activities

These compounds possess many biological properties; however, the influence of the time of collection of the leaves on the composition affects the biological activity and pharmacological applications of the plant. The variation in the constituents in the samples could be attributed to sunlight effect, since all other conditions are the same. The biological activities of

some of the compounds identified as published by a researcher are as summarized in Table 2.

Table 7 shows the effects of ethanol and n-hexane extracts of *P. alliacea* collected at three different times of the day on average weight of rats over two weeks of administration (treatment) compared to acclimatization weeks (pre-treatment) average weight respectively. In the first week of acclimatization, the experimental rats in Groups B to G (186.1, 191.8, 189.5, 191.6, 178.2 and 182.3 g respectively) when compared with the Positive control Group A (182.3 g) shows no significant difference ($P > 0.05$). In the second week, the rats in Groups B to G recorded significant difference in weight across the groups. As the plant extracts administration was introduced to the experimental rats in the third week, all *P. alliacea* administered groups recorded further slight decrease in weights across the groups, which continued into the fourth week.

Table 7 shows there were no significant decrease in the average weight of rats administered with ethanol and n-hexane extracts of *P. alliacea* over two weeks of administration. The slight weight reductions recorded across the weeks of administration per group were not significant enough to cause any harm in the experimental rats. This suggests that the n-hexane and ethanol extracts of *P. alliacea* did not cause any form of damage, swelling or inflation (Sulaiman *et al.,* 2014a).

Table 7: Effects of Ethanol and n-hexane Extracts of *P. alliacea* collected at three different times of the day on Average Weight of Rats over Two Weeks of Treatment vs Pre-treatment.

Groups	**Week one**	**Week two**	**Week three**	**Week four**
A	182.3 ± 19.43^{a}	174.4 ± 17.63^{ab}	176.4 ± 16.57^{ab}	169.7 ± 18.89^{ac}
B	186.1 ± 19.48^{a}	177.6 ± 18.39^{ab}	179.3 ± 17.17^{ab}	173.3 ± 19.03^{ab}
C	191.8 ± 19.48^{a}	182.1 ± 19.10^{ab}	183.6 ± 17.90^{ab}	178.1 ± 20.19^{ac}
D	189.5 ± 17.71^{a}	178.5 ± 16.50^{ab}	180.2 ± 15.97^{ab}	173.2 ± 19.41^{ab}
E	191.6 ± 20.53^{a}	175.7 ± 18.66^{b}	176.8 ± 18.21^{b}	168.2 ± 21.97^{c}
F	178.2 ± 04.96^{a}	171.6 ± 05.37^{a}	172.9 ± 09.95^{a}	168.0 ± 20.47^{ab}
G	182.3 ± 19.43^{a}	174.4 ± 17.63^{ab}	176.4 ± 16.57^{ab}	169.7 ± 18.89^{b}

Values are expressed as mean of three replicates ± S.E.M and those with different superscripts across the group are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea;* (C): Morning n-hexane *P. alliacea*; (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea*)

Table 8 shows the effects of ethanol and n-hexane extracts of *P. alliacea* collected at three different times of the day on weight of organs. For the liver, Kidney and Heart weights, there was no significant difference ($P > 0.05$) between the three control groups (5.62), (0.58), (1.15) and all the treated groups.

Table 8: Effects of Ethanol and n-hexane Extracts of *P. alliacea* on weight of organs

Groups	**Liver**	**Heart**	**Kidney**
A	5.62 ± 0.62^{a}	0.58 ± 0.06^{a}	1.15 ± 0.09^{a}
B	5.56 ± 0.72^{a}	0.61 ± 0.07^{a}	1.19 ± 0.06^{a}
C	5.60 ± 0.72^{a}	0.62 ± 0.08^{a}	1.17 ± 0.06^{a}
D	5.75 ± 0.74^{a}	0.65 ± 0.08^{b}	1.18 ± 0.07^{a}
E	5.67 ± 0.71^{a}	0.68 ± 0.08^{b}	1.19 ± 0.06^{a}
F	5.50 ± 0.80^{a}	0.70 ± 0.08^{b}	1.20 ± 0.07^{a}
G	6.33 ± 0.33^{b}	0.57 ± 0.01^{a}	1.10 ± 0.01^{a}

Values are expressed as mean of three replicates ± S.E.M and those with different superscripts down the group are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea;* (C): Morning n-hexane *P. alliacea*; (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea*)

Organ-body weight ratio is a useful marker of cellular swelling, atrophy or hypertrophy (Amresh *et al.,* 2008; Sulaiman *et al*., 2017). Therefore, the absence of an increase in the computed organ-body weight ratios (Table 8) and lack of significant decrease in organ weights recorded (Table 9) suggests that the n-hexane and ethanol extracts of *P. alliacea* did not cause any form of swelling, atrophy and hypertrophy on the organs of interest within the experimental rats as previous studies (Sulaiman *et al.,* 2015; Oluyomi *et al*., 2016) have associated changes to body weight with toxicity of the drugs, chemicals or extracts administered.

Table 9 shows the effects of ethanol and n-hexane extracts of *P. alliacea* collected at three different times of the day on Organ/Body-Weight ratio. For the liver, heart and kidney, there was no significant difference ($P > 0.05$) between the three Control groups (3.45), (3.59), (0.71) respectively and all the other treated groups (B to G down the group).

Table 9: Effects of Ethanol and n-hexane Extracts of *P. alliacea* collected at three different times of the day on Organ Body-Weight ratio

Groups	Liver	Heart	Kidney
A	3.50 ± 0.52^{a}	0.36 ± 0.05^{a}	0.71 ± 0.09^{a}
B	3.43 ± 0.65^{a}	0.38 ± 0.06^{a}	0.73 ± 0.27^{a}
C	3.34 ± 0.52^{a}	0.37 ± 0.07^{a}	0.70 ± 0.09^{a}
D	3.36 ± 0.56^{a}	0.38 ± 0.07^{a}	0.69 ± 0.10^{a}
E	3.39 ± 0.54^{a}	0.41 ± 0.08^{a}	0.71 ± 0.11^{a}
F	3.44 ± 0.59^{a}	0.44 ± 0.09^{a}	0.75 ± 0.12^{a}
G	3.97 ± 0.37^{b}	0.35 ± 0.03^{a}	0.66 ± 0.09^{a}

Values are expressed as mean of three replicates ± S.E.M and those with different superscripts down the group are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea;* (C): Morning n-hexane *P. alliacea*; (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea*).

Aspartate Transaminases (AST, E.C. 2.6.1.1)

Figure 1 shows the AST Activity (U/I) in the serum of rats administered with n-hexane and ethanol extracts of *Petiveria alliacea*. The AST Activity was significantly higher ($P < 0.05$) in the serum of rats administered with Afternoon n-hexane, Evening Ethanol extracts and Evening n-hexane extracts of *P. alliacea* in Groups E, F and G with a

concurrent decrease ($P < 0.05$) in AST activity in the hearts of rats in the same groups.

The AST activity in the heart of rats administered with three of the extracts in Groups B, C and D when compared with the control Group A shows a significant increase ($P < 0.05$) as shown in figure 1.

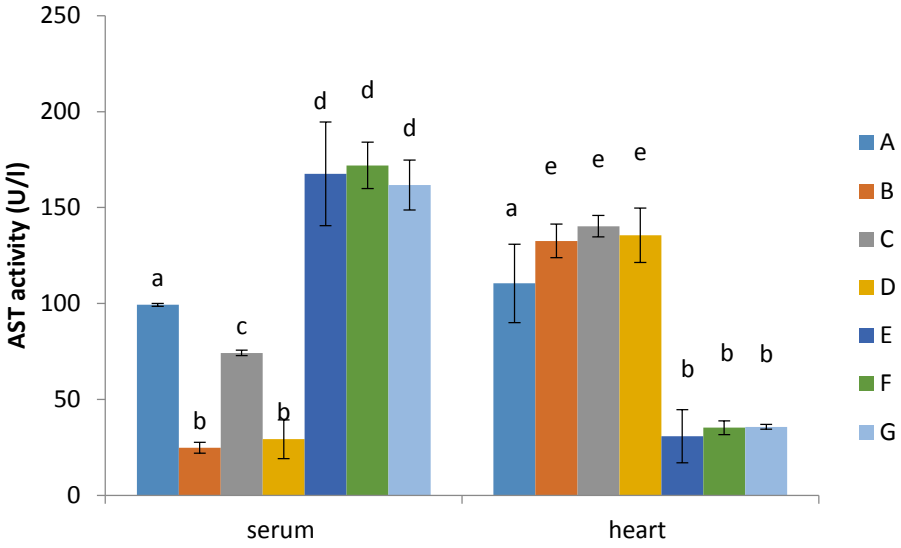

Figure 1: Effects of ethanol and n-hexane extract of *Petiveria alliacea* leaf on AST activity in Wistar rats. Values are mean ± SEM of 3 replicates and those with different alphabets on the bars are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea* C): Morning n-hexane *P. alliacea;* (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea.*

However, the AST activity was significantly reduced ($P < 0.05$) in the serum of rats administered, Morning Ethanol *P. alliacea* (Group B): Morning n-hexane *P. alliacea;* (Group C) and Afternoon Ethanol *P. alliacea* (Group D) when compared with the control (Group A).

Alanine Transaminases

Figure 2 shows the ALT Activity (U/I) in the serum of rats administered with the six leaf extracts of *Petiveria alliacea*. No significant difference (P

> 0.05) in ALT Activity was recorded in the serum of all rats administered with various extracts of *P. alliacea* in Groups B to G when compared to the control (Group A). Similarly, The ALT activity in the liver of rats administered with the six extracts in Groups B to G when compared with the control Group A shows no significant increase (P > 0.05).

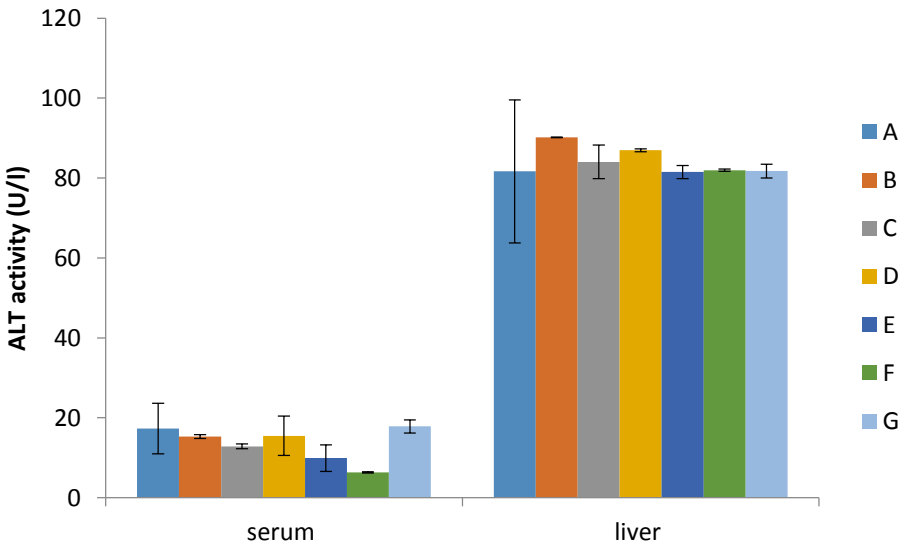

Figure 2: Effects of ethanol and n-hexane extract of *Petiveria alliacea* leaf on ALT activity in Wistar rats. Values are mean ± SEM of 3 replicates and those with different alphabets on the bars are significantly different at (P < 0.05). (A): Control; (B): Morning Ethanol *P. alliacea* (C): Morning n-hexane *P. alliacea;* (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea.*

Alkaline Phosphatase (ALP, E.C. 3.1.3.1)

Figure 3 shows the ALP Activity (U/I) in the serum, liver and kidney of rats administered with n-hexane and ethanol extracts of *Petiveria alliacea*. The ALP activity in the serum of rats administered with the six extracts were significantly lower (P < 0.05) in all the groups than the control. However, the ALP activity was significantly reduced (P < 0.05) in the serum of rats administered with the last three extracts in Groups E, F, and G than the first three groups; B, C and D.

The ALP Activity in the liver of rats administered with extracts in Groups B, C, D, and E when compared with the control Group A shows no significant difference ($P > 0.05$) (figure 3). However, the ALP Activity was significantly reduced ($P < 0.05$) in the liver of rats administered with the last two extracts in Groups F and G when compared with the control group.

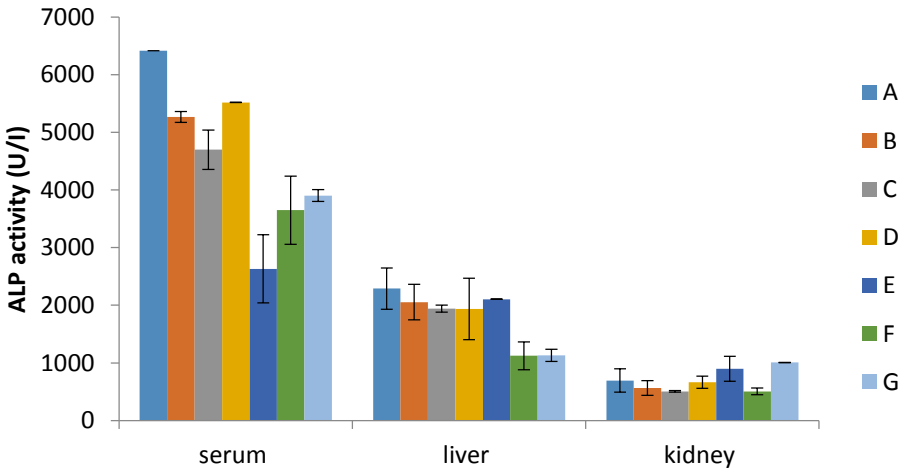

Figure 3: Effects of ethanol and n-hexane extract of *Petiveria alliacea* leaf on ALP activity in Wistar rats. Values are mean ± SEM of 3 replicates and those with different alphabets on the bars are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea* (C): Morning n-hexane *P. alliacea;* (D): Afternoon Ethanol *P. alliacea:* (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea.*

Also, the ALP activity in the kidney of rats administered with extracts in Groups B-G (666, 687, 694, 694, 684, 681 and 690) when compared with the control Group A(668) shows no significant difference ($P > 0.05$) (figure 3).

Albumin

Figure 4 shows the albumin concentration (mg/dl) in the serum of rats administered with n-hexane and ethanol extracts of *Petiveria alliacea*. The albumin concentration (mg/dl) in the serum of rats administered with

extracts in Groups B, C, D when compared with the control Group A shows no significant difference ($P > 0.05$). However, the albumin concentration was significantly higher ($P < 0.05$) in the serum of rats administered with extracts in Groups E, F and G when compared with the control (group A).

The albumin concentration (mg/dl) in the liver of rats administered with extracts in Groups C and F when compared with the control Group A are significantly higher ($P < 0.05$), while no

significant difference ($P > 0.05$) was recorded in the albumin concentration of the liver of rats administered with extracts in Groups B, D, E and G when compared with the control (group A).

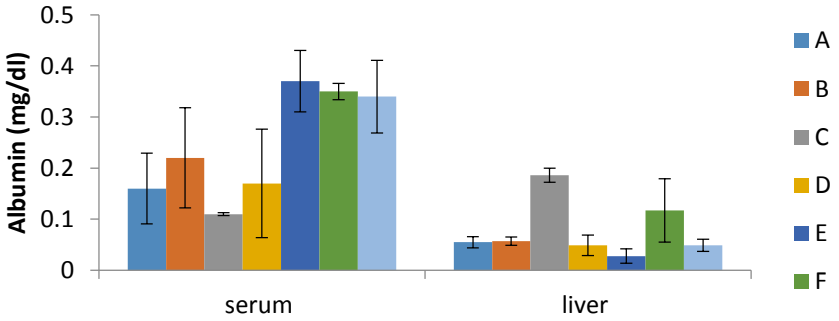

Figure 4: Effects of ethanol and n-hexane extract *of Petiveria alliacea* leaf on albumin concentration in serum and liver of Wistar rats. Values are mean ± SEM of 3 replicates and those with different alphabets on the bars are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea* (C): Morning N-hexane *P. alliacea;* (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea* F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea.*

Urea

Figure 5 shows the urea concentration (mg/dl) in the serum and kidney of rats administered with n-hexane and ethanol extracts of *Petiveria alliacea*. The urea concentration (mg/dl) in the serum of rats administered with the two extracts in Groups C to G (12.1, 12.6, 13.5 11.9 and 13.5) were

significantly reduced ($P < 0.05$) but no significant decrease in group B (24.9) when compared with the control Group A (28.5).

The urea concentration (mg/dl) in the kidney of rats administered with extracts in Groups B to F (13.2, 12.7, 15.6, 14.9, 16.7) when compared to control Group A (34.3) showed a significant decrease ($P < 0.05$) while no significant decrease was recorded in the kidney of rats in Group G.

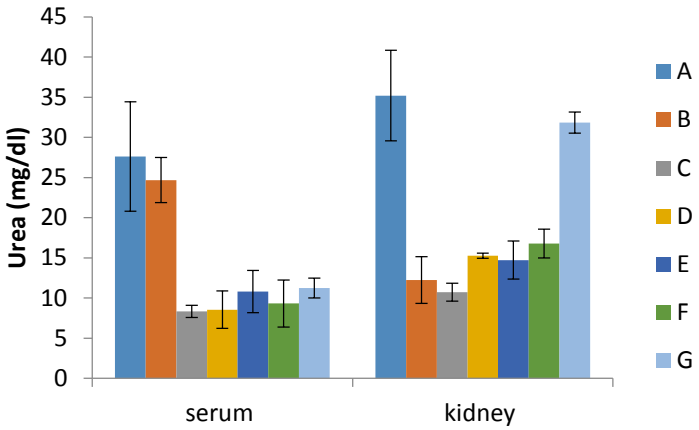

Figure 5: Effects of ethanol and n-hexane extract of *Petiveria alliacea* leaf on urea concentration in serum and kidney of Wistar rats. Values are mean ± SEM of 3 replicates and those with different alphabets on the bars are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea* (C): Morning n-hexane *P. alliacea;* (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea:* (G): Evening n-hexane *P. alliacea*

Total Protein Concentration

Figure 6 shows the protein concentration (mg/ml) in the liver of rats administered with the extracts in all the Groups were significantly reduced ($P < 0.05$) when compared with the control Group A. However the protein concentration (mg/ml) ($P < 0.05$) in the liver of rats administered with extracts in Groups D, E and G were significantly higher ($P < 0.05$) than those recorded in Groups B, C and F, when compared with the control A.

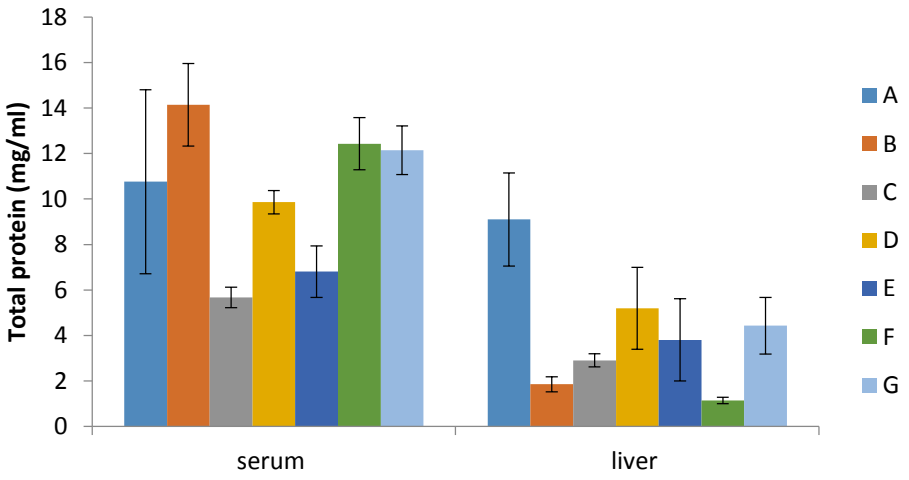

Figure 6: Effects of ethanol and n-hexane extract of *Petiveria alliacea* leaf on total protein concentration in serum and liver of Wistar rats. Values are mean ± SEM of 3 replicates and those with different alphabets on the bars are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea* (C): Morning n-hexane *P. alliacea;* (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea*.

Creatinine Concentration

Figure 7 shows the creatinine concentration (mg/dl) in the kidney of rats administered with n-hexane and ethanol extracts of *Petiveria alliacea*. The creatinine concentration (g/dl) in the kidney of rats administered with extracts in Groups B-G (8.3, 8.1, 7.9, 7.5, 6.9, 7.2) when compared with the control Group A (8.9) was significantly reduced ($P < 0.05$).

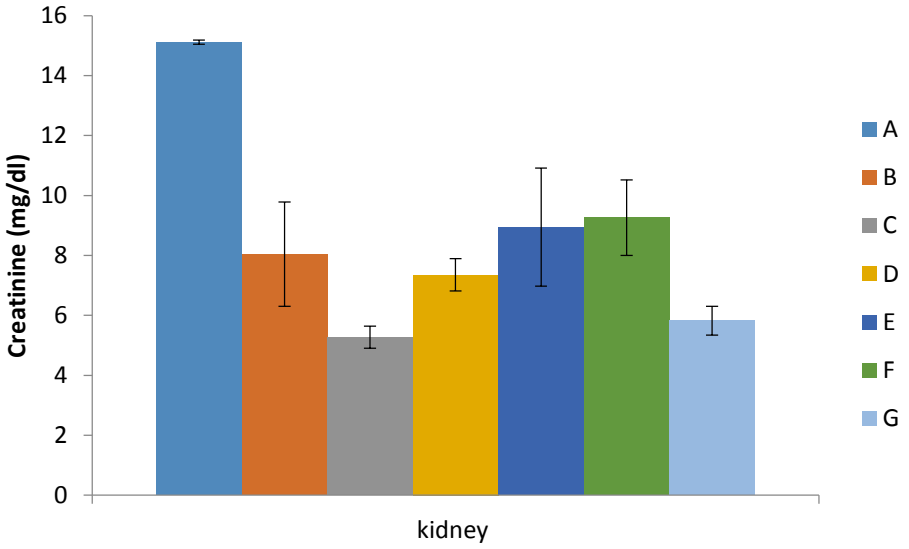

Figure 7: Effects of ethanol and n-hexane extract of *Petiveria alliacea* leaf on creatinine concentration in kidney of Wistar rats. Values are mean ± SEM of 3 replicates and those with different alphabets on the bars are significantly different at ($P < 0.05$). (A): Control; (B): Morning Ethanol *P. alliacea* (C): Morning n-hexane *P. alliacea;* (D): Afternoon Ethanol *P. alliacea*; (E): Afternoon n-hexane *P. alliacea*; (F): Evening Ethanol *P. alliacea*; (G): Evening n-hexane *P. alliacea*.

Discussion

Aspartate Transaminase (AST) Activity (U/I) in the Serum and Heart of Rats Administered Aqueous Extracts of *Petiveria alliacea* collected at three different times of the day.

The aminotransferases (ALT and AST) are 'markers' of liver and heart damage and can thus be used to assess liver and heart cytolysis (Pramyothin *et al.*, 2006; Oluyomi *et al.*, 2016). The high AST activity recorded in the serum of rats administered with Afternoon n-hexane, Evening Ethanol extracts and Evening N-hexane extracts of *P. alliacea* in Groups E, F and G with a concurrent decrease ($P < 0.05$) in AST activity of their hearts may be as a result of the rats' adaptive mechanism in order to offset the stress imposed by the extracts' administration (Sulaiman *et al.*, 2015).

The reduced AST activity in the serum of rats administered Morning Ethanol *P. alliacea* (Group B): Morning N-hexane *P. alliacea;* (Group C) and Afternoon Ethanol *P. alliacea* (Group D) when compared with the control (Group A, which shows that AST may not have leaked from the heart into the serum, implying no damage to the cardiac membranes no toxicity.

Alanine Aminotransferase Activity (ALT) (U/I) in the Serum and Liver of Rats Administered Aqueous Extracts of *Petiveria alliacea* collected at three different times of the day.

The measurement of ALT Activity in human serum has proven to be a valuable indicator of liver function in clinical settings (Huang *et al.*, 2006). The aminotransferases (ALT and AST) are 'markers' of liver damage and can thus be used to assess liver cytolysis with ALT being a more sensitive biomarker of hepatotoxicity than AST (Pramyothin *et al.*, 2006). The insignificant increase recorded in ALT Activity in the serum of all rats administered the various extracts of *P. alliacea* in Groups B to G as well as the no significant reduction in hepatic ALT activity implies that the integrity of the hepatic membranes were preserved (Oluyomi *et al*., 2016; Sulaiman *et al.,* 2017) as no leakage has occurred from the liver into the serum.

Alkaline Phosphatase Activities of the Serum and Selected Tissues of Rats Administered Aqueous Extracts of *Petiveria alliacea* collected at three different times of the day.

ALP is present in all tissues throughout the body, but is particularly concentrated in liver, bile duct, kidney, bone, intestinal mucosa and the placenta (Tamas *et al.,* 2002). ALP is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum (Wright and Plummer, 1972) and is often used to assess the integrity of the plasma membrane and endoplasmic reticulum (Akanji *et al.,* 1993; Sulaiman *et al.,* 2015; Oluyomi *et al*., 2016)

The reduced ALP activity in the serum of rats administered with the six extracts in all the groups is a positive indication of little or no toxicity as an increase may have been as a result of membrane compromise which would have allowed ALP's leakage from other tissues into the serum. The

last three extracts administered to Groups E, F, and G are therefore, even safer for consumption than the first three extracts in Groups B, C and D.

A reduced trend was recorded in the liver with no significant difference recorded in the ALP activity in the liver of rats administered with extracts in Groups B, C, D, and E as well as more reduction in the ALP Activity recorded in the liver of rats administered with the last two extracts in Groups F and G. Also, the low ALP activity in the kidney of rats administered with extracts in Groups B-G may not be unconnected with leakage of ALP from the kidney into the serum,

resulting in the high ALP activity initially recorded in the serum, which still queries the level of safety achievable with the consumption of the extracts.

Albumin Concentration (g/dl) in the Serum and Liver of Rats Administered Aqueous Extracts of *Petiveria alliacea* collected at three different times of the day.

The level of albumin has been associated with the synthetic function of the liver (Adeyemi *et al*. 2010, 2012). Albumin is the major plasma protein with molecular weight of approximately 66.3kDa. It is synthesized exclusively in the liver. The concentration of albumin is a useful 'marker' of secretory, synthetic and excretory functioning of the liver and kidney (Yakubu and Musa, 2012; Sulaiman *et al.*, 2014a). A falling concentration in chronic liver disease suggests a clinically significant deterioration in liver function 'decompensation'.

There was no significant albumin concentration (g/dl) recorded in the serum of rats administered with extracts in Groups B, C, D when compared with the control Group A shows that these three extracts are safe for consumption as they do not alter significantly, the serum albumin concentration of the host.

However, the increased albumin concentration recorded in the serum of the remaining rats administered with extracts in Groups E, F and G when compared with the control (group A) may imply that the membranes of the liver have been ruptured. Similarly, the albumin concentration in the liver of rats administered with extracts in Groups C and F is high, which may

be an indication that more plasma protein has been synthesized (Yakubu and Musa, 2012; Sulaiman *et al.,* 2017a).

Urea Concentration in the Serum and Kidney of Rats Administered Aqueous Extracts of *Petiveria alliacea* collected at three different times of the day.

Serum urea is one of many useful parameters for evaluating the status of real function. An increase in the level of serum urea may imply renal excretion (Adeyemi and Akanji, 2012). The significance of the enzyme urease includes: to serve as a virulence factor in human and animal infections of the urinary and gastrointestinal tracts, play role in recycling of nitrogenous wastes in the rumens of domestic livestock (Mobley and Hausinger, 1989; Sulaiman *et al.,* 2017b). In this study, the reduced urea concentration (mg/dl) recorded in the serum of rats administered with the two extracts in Groups C-G after the administration of the first five extracts may be attributed to decreased amino acid degradation by the liver. A similar reduction in urea concentration (g/dl) in the kidney of rats administered with extracts in Groups B-G was also recorded except for Group G where no significant increase was recorded.

Protein Concentration in the Serum and Liver of Rats Administered Aqueous Extracts of *Petiveria alliacea* collected at three different times of the day.

The concentration of total protein is a useful 'marker' of secretory, synthetic and excretory functioning of the liver (Sulaiman *et al.,* 2017; Yakubu and Musa, 2012). The total protein is composed of albumin and globulin and reflects the balance of protein biosynthesis and catabolism.

The no significant difference recorded for protein concentration (mg/ml) in the serum of rats administered with extracts in Groups B, D, F and G is a positive safety indication, while the reduced protein concentration (mg/ml) recorded in the serum of rats administered with two of the extracts in Groups C and E may be as a result of decreased protein synthesis, increased protein loss, increased catabolism or protein malabsorption, consequent upon the extracts' administration.

The protein concentration (mg/ml) in the liver of rats administered with the extracts in all the Groups were significantly reduced ($P < 0.05$) when

compared with the control Group A. However, the protein concentration (mg/ml) ($P < 0.05$) in the liver of rats administered with extracts in Groups D, E and G were significantly higher ($P < 0.05$) than those recorded in Groups B, C and F, when compared with the control. Improved protein synthesis with concomitant reduction in loss of protein may have contributed to this.

Creatinine Concentration in the Kidney of Rats Administered Aqueous Extracts of *Petiveria alliacea* collected at three different times of the day.

Creatine is derived from creatine and creatine phosphate in muscle tissue and may be defined as a nitrogenous waste product. Creatitine is not reutilized but is excreted from the body in the urine via the kidney. During the reaction involving creatine and phosphocreatine, catalyzed by creatine kinase, spontaneous conversion to creatine might occur (Allen, 2012). A rise in blood creatinine levels is observed only with marked damage to functioning nephrons. The reduction in creatinine concentration (mg/dl) in the kidney of rats administered with extracts in Groups B to G when compared with the control Group A suggests an enhanced glomerular clearance rather than an impaired muscle metabolism.

Conclusion

It can be concluded that *Petiveria alliacea* leaves contain biologically active components that may be responsible for the folklore and scientifically documented medicinal effect of the plant and also that the time of collection of the leaves have significant effect on the chemical composition and hence the biological activity of the plant from the results generated in this research. Biochemical results obtained generally suggest that, the aqueous extracts of *Petiveria alliacea* leaves collected at three different times of the day showed alterations, both positive and negative, toxicological effects. Conclusively, it can be inferred that leaf extract of *P. alliacea* has the tendency to rupture the tissues' membrane in their hosts if taken in excess. Hence, care must be taken by the public from the excessive or erratic consumption of leaf extracts of *P. alliacea* for agricultural and medicinal purposes.

Acknowledgements

We wish to acknowledge the efforts of the following students (Egousa EO and Ajape BA) in the source and collection of the samples. Also, the authors appreciate the technical staff of Chemistry and Biochemistry Departments for their assistance during the bench work of this research.

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