Qualitative and Quantitative Evaluation of Phytochemical Constituents of Leaf and Root of *Petiveria alliacea* Using Spectrophotometric Technique

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Abstract

The present study investigates the qualitative and quantitative analysis of phytochemical constituents of *Petiveria alliacea* root and leaf using methanol and n-hexane as a solvent. Qualitatively all phytochemicals were present but the different concentrations at which they were present were determined using spectrophotometric method.

The methanol leaf extract revealed that the trypsin inhibitor was the most abundant (7.02 mg/g ± 0.09) followed by steroids (4.59 mg/g ± 0.01), oxalate (4.24 mg/g ± 0.01), saponins (2.39 mg/g ± 0.005 and flavonoid (2.03 mg/g ± 0.01) while alkaloids were the least abundant (0.001 mg/g ± 0.0001). However the root extract revealed that the oxalate content was the highest (4.22 mg/g ± 0.0), followed by the steroids (3.28 mg/g ± 0.01), terpenes (2.7 mg/g ± 0.05) and phenols (2.28 mg/g ± 0.02) with alkaloids being the least present (0.001 mg/g ± 0.0002).

In the hexane extract of the leaves, steroids were the most abundant (4.59 mg/g ± 0.02) followed by oxalate (3.57 mg/g ± 0.02), terpenes (2.60 mg/g ± 0.01), and flavonoids (2.03 mg/g ± 0.02) while alkaloids (0.003 mg/g ± 0.0002) were the least abundant. The root extract of hexane had oxalate to be the most abundant (4.59 mg/g ± 0.02), followed by steroids (3.94 mg/g ±0.05) and flavonoids (2.52 mg/g ± 0.02) with alkaloids again being the least abundant (0.014 mg/g ± 0.002).

The overall results of the study suggest that *Petiveria alliacea* is a good source of therapeutic compounds due to the various phytochemicals present in it.

Keywords: Phytochemicals, Quantitative, Qualitative, Hexane, Methanol, *Petiveria alliacea*, Therapeutic.

INTRODUCTION

Medicinal plants are plants with the ability to synthesize chemical compounds used to perform various important biological functions within the plants and to defend them against predators (Tapsell *et al*., 2006). As part of traditional healthcare, medicinal plants have been used in most parts of the world for thousands of years (Ajayi *et al*., 2010). The use of these plants by man in the treatment of ailments and diseases is a common practice in the developing countries.

Petiveria alliacea also called Anamu, Congo root, Apacin, Garlic weed because of the strong garlic smell of its root (Technical data report for Anamu 2002) and Pejoporo by the Yoruba natives of Nigeria happens to be one of such medicinal plants used in the treatment of various ailments. It belongs to the Phytolaceae family and grows in the Caribbean, Latin America, Africa and other regions of the world. *Petiveria alliacea* has been used in traditional medicine for pain relief, as anti-cancer, anti-viral, anti-tumor, anti-inflammatory, anti-influenzer, anti-bacterial and as anti-fungal drug to mention a few (Tropical Plant Database-Anamu).

Several pharmacological studies have been conducted to confirm its properties and results shows that it has anagestic and anti-inflamatory activities (Germano *et al.,*1995, Lopes-Martins et al., 2002), aids uterine contraction (Oluwole *et al*., 1998) and has anti-sickling properties (Adejumo et al., 2011). All of these properties can be said to be due to the presence of some chemicals constituents in plants which are referred to as phytochemicals.

Phytochemicals are bioactive chemicals which produce a definite physiological action on the human body. Some of these important bioactive constituents are alkaloids, tannins, flavonoids and phenolic compounds. (Hill, 1952, Edeoga *et al*., 2005).

Several chemical tests have been carried out on both aqueous and alcoholic extract of plants in order to identify its chemicals constituents using standard procedures. However this study explores the use of the Ultra violet spectroscopy in quantifying phytochemicals present in *Petiveria alliaceae* leaf and root while standard procedures were used for the qualifying tests as described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973).

MATERIALS AND METHOD

Plant collection and preparation

Whole plant of *Petiveria alliaceae* was harvested from a personal garden in Ijebu-Ode, Ogun State, South-Western Nigeria. The plant was identified at the herbarium of the Botany Department of University of Ibadan. The plant was properly rinsed with distilled water to get rid of sand particles after which the leaves and the root were plucked out and air dried at room temperature for eight (8) weeks. The dried leaves and root were pulverized and stored in airtight polythene bags and labeled for further study.

Extraction

100 g each of the pulverized leaves and root of *Petiveria alliaceae* was soaked in 500 ml of methanol and n-hexane for 24 hours. A greenish (dark) paste like was obtained. The mixture was filtered and separated using separating funnel. The filtrates was concentrated using rotary evaporator i.e. methanol and n-hexane extracts and residue preserved for further use.

Phytochemical screening

Qualitative determination of phytochemical constituents in *Petiveria alliaceae* leaves and roots.

Chemical test were carried out on the both the grinded samples and methanolic extracts for the qualitative determination of phytochemical constituents of *Petiveria alliaceae using* standard procedures as described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973).

Quantitative determination of phytochemical constituents in *Petiveria alliaceae* leaves and roots.

The quantitative determination was achieved on both methanol and n-hexane extracts with the aid of the UV-Visible spectrophotometer as described below for each phytochemical being investigated.

Saponins

2 g each of the crude extract of the leave and root was weighed into a 250 mL beaker and 100 ml of isobutyl alcohol was added and left for 5hours on a UDY shaker for uniform mixing so as to obtain a homogenous solution. The mixture was then filtered using a filter paper. The filtrate was transferred into another beaker and saturated with magnesium carbonate solution.

The mixture obtained was again filtered to get a clear colorless solution which is then read on the spectrophotometer at 380nm.

Phenol

1 g of each extract was weighed into a 250 mL conical flask and 20 mL of distilled water was added to it. The solution was filtered after 4 days into a 100 mL standard flask and made up to the mark with distilled water. 1 mL of the filtrate from each sample (leaves and root) was measured into a test tube and 3 ml each of 0.008 M potassium hexacyanoferrate (III) and 0.01 M of iron (III) chloride were added into each filtrate. The absorbance of each filtrate was then taken after 10 minutes at 760 nm.

Tannin

1 g of the extracts were weighed into a 250 mL beaker and soaked with a mixture of acetone (80 mL) and glacial acetic acid (20 mL) for 5 hours to effect extraction of the tannin. The solution was filtered through a double layer of filter paper so as to obtain optimum filtration. A set of standard solution of tannic acid was prepared ranging from 10 ppm to 50 ppm. The absorbance of the standard as well as that of the filtrate was then read at 500 nm.

Terpenes

0.05 g of the samples were weighed into 50 mL conical flask, 20 mL of chloroform-methanol mixture in the ratio 2:1 was added, shake thoroughly and allowed to stand for 15 minutes. The mixture was later centrifuged for 15 minutes to obtain a supernatant and a precipitate. The supernatant was discarded and the precipitate was rewashed with another 20 mL of the chloroform-methanol mixture for re-centrifugation. The resultant precipitate was dissolved in 40 mL of 10% Sodium Deodocyl Sulphate solution. 1 mL of 0.01 M Ferric Chloride solution was added to the above at 30 seconds interval, shaken well and allowed to stand for 30 minutes. Standard terpenes ranged 0-5mg/ml were prepared from 100 mg/L stock terpene solution obtained from Sigma-Aldirch chemicals, USA. The absorbance of the sample as well as that of the standard concentrations of Terpenes were read on a digital spectrophotometer at a wave length of 510 nm.

Flavonoids

1 g of the sample was weighed into 250 mL conical flask. 20 mL of warm distilled water was added to it and it was then placed inside a water bath at 100^{o}C for 10 minutes. The solution was then filtered and 1 mL of the filtrate was pipette into a clean test tube with 1ml of 0.5 M NaOH added. 8 ml of distilled water was added to the resulting solution and it was allowed to stand for 10 minutes. The absorbance was read at 410 nm.

Oxalate

1 g of each sample was weighed into a 250 mL conical flask and soaked with 100 mL of distilled water. The mixture was allowed to stand for 3 hours and each sample was filtered. 10 – 50 ppm standard solution of oxalic acid was prepared and read on the spectrophotometer at 420nm for the absorbance.

Steroids

20 mL of chloroform-methanol mixture in the ratio 2:1 was added to 0.5 g of the sample extract in 100 mL beaker with shaking for 30 minutes in other to dissolve the extract, the mixture was then filtered.

The resultant residue was repeatedly treated with chloroform-methanol mixture until it was ascertained free of steroids. 1 mL of the filtrate was pipette into a test tube and 5 mL of alcoholic KOH was added then shaken thoroughly to obtain a homogenous mixture. The mixture was later placed in in a water bath set at 37ºC-40ºC for 90 minutes. After 90 minutes, mixture was cooled to room temperature and 10 mL of petroleum ether was added followed by 5 mL distilled water and the whole mixture was evaporated to dryness on a water bath.

To the dried residue was 6 ml Liebermann Buchard reagent added in a dry bottle and the absorbance taken at a wavelength of 620 nm.

Trypsin inhibitor

0.2g of the sample was weighed into a screw cap centrifuge tube and 10mLof0.1M phosphate buffer was added to it and the whole content of the tube was shaken at room temperature for 1lour on a UDY shaker. The suspension obtained was centrifuged at 5000rpm for 5minutes. The volume of each sample was adjusted to 2ml with phosphate buffer and the test tubes were placed in water bath which was maintained at 37ºC.

6ml of 5%TCA solutions was added to a control tube (blank) while 2mL of casein solution was added to the other tubes that were maintained at 37ºC. These were incubated for 20 minutes and the reaction was stopped after 20minutes by adding TCA solution to the experimental tubes and shaken. The reaction was allowed to proceed for 1hour at room temperature after which he mixture was filtered through whatmann filter paper. The absorbance of the filtrate from the sample and that of trypsin standard solution were read at 280nm.

Cardiac glycosides

The procedure described by (Sofowora, 1993) was used, 10 ml the of the extract was pipette into a 250 ml conical flask. 50 ml chloroform was added and shaken on vortex mixer for 1hour. The mixture was filtered into 100 ml conical flask. 10 ml of pyridine and 2 ml of 29 % of sodium nitroprusside were added and shaken thoroughly for 10 min. 3 ml of 20 % NaOH was added to develop a brownish yellow colour. Glycosidesstandard (Digitoxin). A concentration which range from 0 – 50mg/ml were prepared from stock solution the absorbance was read at 510 nm.

Alkaloids

2 g of the each sample was weighed into a 250 ml conical flask and 20 ml of 80 % alcohol was added to give a smooth paste. To the mixture was more alcohol added to make it up 100 ml. 1 g of magnesium oxide was added to the mixture and it was digested in a boiling water bath for 1 hour 30 minutes under a reflux air condenser with occasional shaking. While hot the mixture was filtered through a Buchner funnel. The residue was poured back into the flask and digested for 30minutes with 50mL alcohol after which the alcohol was evaporated then hot water was added.

Upon total removal of the alcohol, 2-3 drops of 10 % HCl was added, 5 mL Zinc acetate solution and 5 mL Potassium Ferro cyanide solutions were added to the solution and it was thoroughly mixed to get a homogenous solution.

The flask was allowed to stand for a few minutes thereafter the solution was filtered and the filtrate was transferred into a separating funnel and the alkaloids present were extracted by shaking vigorously for with 30mL of chloroform five successive times. The residue obtained was dissolved in hot water and transferred into a Kjeldahl flash and 0.2 g sucrose, 10 mL concentrated H_2SO_4 and 0.02 g Selenium was added for digestion to obtain a colorless solution which was then read on the spectrophotometer at 420 nm

Phytate.

2 g each of the sample was weighed into a 250 mL conical flask. 100 mL of 2% concentrated HCl was used to soak the samples for 3 hours. The solution was filtered through a double layer of filter paper then 50 mL each of the filtrate was placed in a 250 ml beaker and 107 mL of distilled water added to each to ensure appropriate acidity. 10 ml 0f 0.3 % Ammoniun thiocyanate solution was added to each solution as indicator. The resulting solution was then filtered and a standard iron (III) chloride solution containing 0.00195 g of iron per mL was. The end point was slightly brownish-yellow and this color persisted for 5 minutes. It was then read on the spectrophotometer at 450 nm.

Statistical Analysis

All results are expressed as mean ± standard deviation. All results are means of triplicates and the level of statistical significance is expressed at $p< 0.05$.

RESULTS AND DISCUSSION

The result of the qualitative phytochemical screening of the plant sample is shown in table 1. The result shows that the plant (*Petiveria alliacea*) contains tannins, phenols, alkaloids, phytates, oxalates, steroids saponins, flavonoids, terpenes, cardiac glycosides and trypsin inhibitors. This result is in agreement with that of other researchers such as Adejumo *et al.,* 2011 who reported the presence of alkaloids, tannins and saponins in the plant of study. Mulyani *et al.,* 2012 also noted the presence of alkaloid, flavonoids and tannins in ethanol extract of *P. alliacea*. Darllen *et al.,* 2013 in their findings also reported the presence of acids, phenols and tannins in the young branches of the plant and alkaloids, steroids, triterpenoids, saponins, phenols and tannins in the leaves of the same plant cultivated in Brazil most of which were all found present in this study.

Table 1: *Result of qualitative analysis of phytochemicals in Petiveria alliacea leaves and root*.

Phytochemicals determined	*Petiveria alliacea* leaves	*Petiveria alliacea* root
Tannin	+	+
Phenol	+	+
Alkaloids	+	+
Saponins	+	Trace
Cardiac glycosides	+	+
Oxalate	+	+
Phytate	+	+
Flavonoids	+	Trace
Terpenes	Trace	Trace

Steroids	+	+
Trypsin inhibitors	+	+

+ = present

Fig. 1 and 2 respectively shows the quantity of each phytochemical present in n-hexane and methanol extracts of the leaves and the root. The analysis shows that some of the phytochemicals vary significantly ($p<0.05$). The methanol extract of the leaves contained considerable high trypsin inhibitors content (7.02 mg/g) compared to other phytochemicals analyzed, followed by steroids which was present in the same quantity (4.59 mg/g) in both hexane and methanol extract of the leaves while the least abundant was the alkaloids (0.0011 - 0.014) mg/g in both plant parts and extraction solvent used.

Saponins are known to have anti carcinogenic properties (Adesokan and Akanji, 2010) hence this justifies the use of *P. alliacea* in the treatment of cancer as documented in PubMed (Kubec et al., 2003). In a study conducted by Adejumo *et al.,* 2011 it was noted that *P. alliacea* has antisickling property and this may be linked to the presence of alkaloids which serve as nerve stimulants, convulsants and muscle relaxants (Kenner and Yves,1996), indicating the use of the plant for the relieve for pains. Flavonoids have been reported to be used as antioxidants or free

radical scavengers (Kar, 2007) hence its presence in *P. alliacea* could be an addition to some of its use as a free radical scavenger. (Tropical plant database)

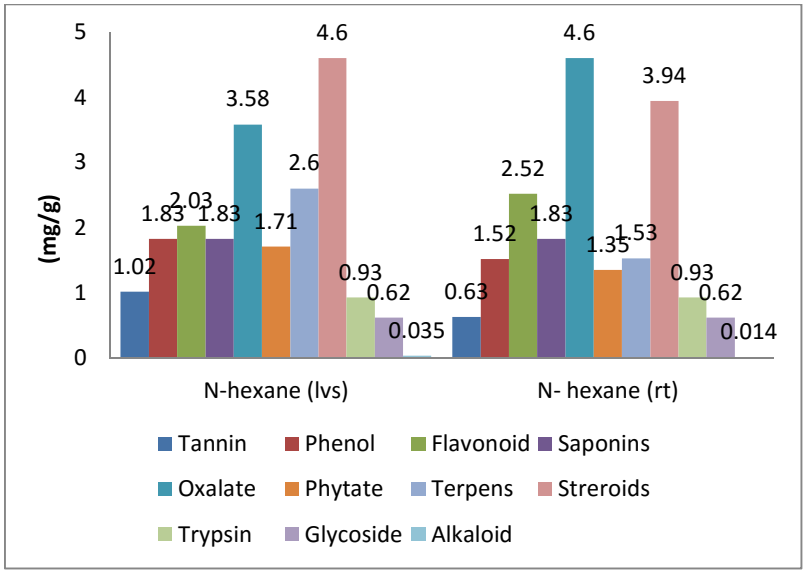

Fig. 1: *Showing graph of the mean values of phytochemicals using N-hexane*

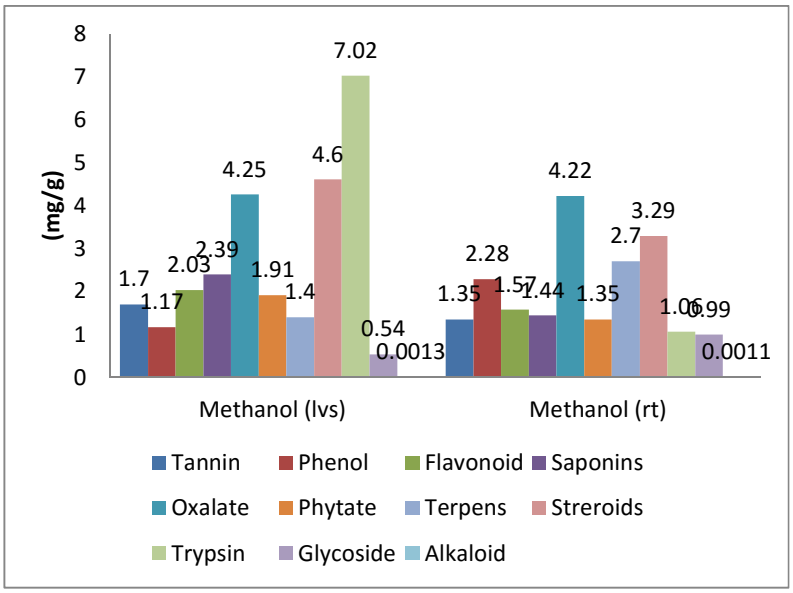

Fig. 2: *Showing graph of the mean values of phytochemicals using methanol*

n-Hex $_{lvs}$ = leave extract of n-hexane, n-Hex $_{rt}$ = root extract of n-hexane,
Methanol$_{lvs}$ = leave extract of Methanol, Methanol$_{rt}$ = root extract of Methanol.

CONCLUSION

The study has revealed that leaves and root of *Petivera alliacea* are rich in phytochemicals (Saponinis, Alkaloids, Flavonoids, Phenolics and Terpenoids among others) which could be the basis for their being used for medicinal purposes. However, caution should be taken in the intake and administration of the leaf and roots extracts because of the saponin and alkaloid content which may induce some side effects. Thus, methanol and hexane are good solvent of extraction for the studied plants.

Toxicology studies should be carried out on both the roots and leaves samples of these plants in order to ascertain the extent or degree of side effects attributable to them. Moreover, further studies should be carried out on mature seeds of this plant species.

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