PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITIES OF THE LEAVES OF PETIVERA ALLIACEA (LINN)

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ABSTRACT

Petiveraalliacea has been widely employed in folkore medicine in the treatment of oxidative stress mediated pathologies such as pain, inflammations as well as a wide range of microbial infections. However, the scientific basis for its observed therapeutic effect is yet to be fully elucidated. Herein, acetone extract of the pulverized air-dried leaves of Petiveraalliacea was tested for its phytoconstituent and antioxidant property. The phytochemical screening (e.g alkaloids, tanins, saponins, flavonoids, etc) was performed using standard procedures. We observed that alkaloids, tannins, phenols, flavonoids and saponins, are predominantly present in the extract. Besides, the determination of antioxidant activity of the extract was done using 2,2- Diphenyl-1-picrylhydrazyl radical (DPPH) using ascorbic acid (Vitamin C) as standard drug, the extract exhibited potent free radical scavenging ability that is comparable to the standard drug (Vitamin C) and this effect may be related to its observed phytoconstituents. The results obtained is in agreement with literature and ethnobotanical survey, this result suggest the presence of antioxidants properties in Petiveriaalliacea and its role in traditional medicine. The antioxidant assay results obtained were expressed as Mean ±SD and the data was analyzed using one-way ANOVA.

Keywords: *Petiveriaalliacea, one-way ANOVA, 2,2- Diphenyl-1-picrylhydrazyl radical (DPPH), phytoconstituent, antioxidant property.*

1.0 INTRODUCTION

PetiveriaAlliacea (linn) is a genus of flowering plants in the pigeon-berry family, It is a deeply rooted herbaceousperennialshrub growing up to 1 m (3.3 ft) in height and has small greenish piccate flowers. The roots and leaves have a strong acid, garlic-like odour which taints the milk and meat of animals that graze on it. *PetiveriaAlliacea (linn)* is used in teas, extracts and capsules, the leaves and the roots are used for medicinal purposes. The common name for petiveraalliacea are: Guinea Henweed, Anamu, Garlic weed and the Yoruba refer to it as ojusaju.

Mild (Mild, 2004) reported that the plant is used to reduce inflammation and pain, it can inhibit the growth and totally destroy the existing strains of bacteria, fungi, candida, arthritis and viruses. Immune system and increase in urination can also be enhanced. The plant has been reported to lower the blood sugar level and to destroy cancer cells (Schmelzer, 2008 and Hernández, 2014). The research is aimed at providing scientific evidence for the claimed therapeutics.

2.0 MATERIALS AND METHODS

Collection and identification of the plant

The leaves of *P. alliaceae* were collected from Ilesha, in Ilesha West Local Government area, Osun State western part of Nigeria and were authenticated at the Obafemi Awolowo University ,Ile –Ife with herbarium voucher number: IFE 17570.

Extraction of Plant Materials

The Leaves of *P. alliacea* were washed to remove dirt's and were air-dried at room temperature (30°C) for 24days; it was then pulverized and kept in an air- tight container until use. 35g of *P.alliacea* was soaked into 350ml of acetone in ratio 1:10 for complete extraction at room temperature (30°C) for 72 hours using cold maceration method. The extract were filtered with Whatmann filter paper No. 42 and then with cotton wool. The residue was discarded and the filtrate was allowed to evaporate to dryness. The percentage yield of the extract was determined. The extract was used for the determination of the phytochemical content and antioxidant property using DPPH methodology.

Phytochemical Screening

Phytochemical screening was performed using standard procedures (Sofowora, 1993) and (Trease *et al.,* 1989)

Test for terpenoids (Salkowski test)

To 0.5 g each of the extract was added 2 ml ofchloroform. Concentrated H_2S0_4(3ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for flavonoids

Five ml of dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. 1ml of concentrated sulphuric acid was added. A yellow colouration that disappears on standing indicates the presence of flavonoids.

Test for tannins

About 0.5g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-blackcolouration

Test for alkaloids

0.5g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml ofacetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer'sreagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for saponins

0.5g of extract was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for cardiac glycosides (Keller-Killianitest)

To 0.5g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ringmay appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for reducing sugars (Fehling's test)

The aqueous ethanol extract (0.5g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

Test for Phenols

Equal volume of extracts solution were filled and mixed together. A deep blue green solution confirmed the presence of phenols.

Test for Resins

2.5ml of copper II sulphate solution was added to 2.5ml of the extract. The resulting solution was shaken vigorously and allowed to settle down, green colour indicates negative test.

Determination of antioxidant activity

The radical scavenging ability of the extract was determined using the stable radical DPPH (2,2-Diphenyl-1-picrylhydrazyl radical) as described by Brand-Williams *et al.*(1995). The reaction of DPPH with an antioxidant compound which can donate hydrogen, leads to its reduction (Blois, 1958). The change in colour from deep violet to light yellow was measured spectrophotometrically at 515nm.

To 1ml of different concentrations(0.05, 0.025, 0.0125, 0.00625, 0.003125, 0.0015625mg/ml) of the extract or standard (vitamin C) in a test tube was added 1ml of 0.3mM DPPH in methanol. The mixture was mixed and incubated in the dark for 30mins after which the absorbance was read at 515nm against a DPPH control containing only 1ml methanol in place of the extract. The percentage inhibition was calculated in the following way:

$$I\% = \frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test extract), and A_{sample} is the absorbance of the test extract. Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration.

Statistical Analysis

The antioxidant assay results obtained were expressed as Mean ±SD. The data was analyzed using one-way ANOVA (Betty, 2003).

3.0 RESULTS

The results of the phytochemical screening of the leaves of *Petiveriaalliacea* are as shown below in Table 1

Table 1: Screening for the phytochemical constituents of *Petiveriaalliacea*

S/N	Phytochemical	Observation
1	Tannis	+++
2	Glycoside	-
3	Resins	-
4	Saponins	++
5	Flavonoids	++
6	Phenols	+++
7	Carbohydrate	-
8	Alkaloids	++
9	Terpeniods	++

Table 2: Antioxidant activity (% inhibition) of Vitamin C and *Petiveriaalliacea*

Concentration (mg/ml)	*P. alliacea* extract (%)	Vitamin C (%)
5.000	0.6160±0.0365	0.1123±0.0005
2.500	0.7640±0.0236	0.2406±0.0037
1.250	0.8270±0.0043	0.3666±0.0030
0.625	0.8810±0.0010	0.4773±0.0072
0.600	0.9500±0.0011	0.5436±0.0081
0.550	0.1000±0.0017	0.6183±0.0020

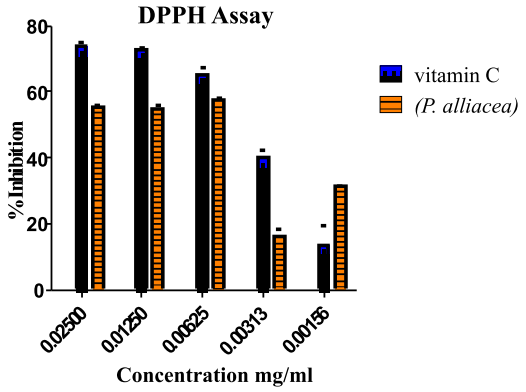

Figure 1: Antioxidants activity (% inhibition) of the vitamin C and Petiveriaalliacea.

4.0 DISCUSSION

The screening of phytochemical constituents of Petiveriaalliacea reveal that the presence of tannis, flavonoids, phenols, saponins, terpeniods and alkaloids are in abundance and in moderate amount as presented in table 1. These phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (Ayodele *et al.,* 2015).The phytochemicals present are responsible for the medicinal value attributed to *P. alliacea*. The presence of phenols, flavonoids and tannis in abundance suggest that the plant can scavenge free radicals that are present in its environment (Ayoola, 2008);these phytochemicals present are responsible for the significant anti-oxidant activity exhibited by *P. alliance.* The IC_{50} of 0.025ug/ml was recorded for *Petiveriaalliacea* and an IC_{50} of 0.0046ug/ml was recorded for Vitamin C, the IC_{50} value for *P. Alliacea* shows that it exhibit strong anti-oxidant activities, it means at a concentration of 0.025ug/ml the plant will scavenge 50% of free radicals present in its environment, and the IC_{50} of *P. alliacea* and Vitamin C are comparable.

The percentage scavenging activity of*P. alliacea* is comparable to that of Vitamin C at a concentration of 0.00625mg/ml and at a concentration of 0.00156mg/ml the percentage scavenging activity of *P. alliacea* was higher than the standard drug (vitamin C). Ethnobotanical survey shows that P.alliacea had been used to treat arthritis and eliminate cancer cells which are predominantly caused by excess free radicals that are in the body and P.alliacea had the capacity to scavenge those excess free radicals in the body (Schmelzer *et al.,* 2008 and Hemandez *et al.,* 2014)

The presence of flavonoids, tannins and phenol in P.alliacea confirms that the plant is highly phenolic and phenolic compounds are a major group of compounds that acts as primary antioxidants or free radicals scavengers(Ayoola*et al.,* 2008).

5.0 Conclusion and Recommendation

The result suggests that *P. Alliacea* contains active ingredients that can control and scavenge free radicals that are present in the body. Therefore this result is in agreement with literature review and traditional use of the plant as an active plant capable of eliminating cancer cells and treating of arthritis. Toxicity studies should be carried out to ascertain the presence and extent of any toxic compounds that may be found due to the presence of saponins and alkaloids. Further studies should be done to quantify the phytochemicals present and bio-guided fractionation can be employed to isolate and characterize the bioactive compounds in the plant.

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