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Phytochemical Profile and *In vitro* Antioxidant Capacity of *Petiveria alliacea* Plant Parts Extract

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Plants have significantly contributed to the discovery of modern drugs that have therapeutic potential. This study was carried out in order to evaluate the phytochemical constituents and *In vitro* antioxidant potential of the leaf and root of *Petiveria alliacea* extracts. The aqueous and ethanolic leaf and root extracts of *Petiveria alliacea* were screened by qualitative and quantitative analysis. Different concentrations of the aqueous and ethanolic extracts were scanned within the wavelength range of 378–654 nm. The *in vitro* antioxidant (catalase and hydrogen peroxide scavenging) potential of the extracts was determined spectrophotometrically. The study revealed the presence of tannins, phlobatannins, saponins, flavonoids, terpenoids, and cardiac glycosides in aqueous and ethanolic extracts of the leaves and root. High levels of total alkaloids and flavonoids were observed in the ethanolic and aqueous leaf extracts. The ultraviolet-visible profile of the extracts revealed high peaks at 378 nm for the leaf and root at different concentrations, confirming the

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presence of flavonoids and alkaloids in the plant. High hydrogen peroxide scavenging activity was observed in aqueous extracts of the leaf and root, and in the ethanolic leaf extract but not in the ethanolic root extract. The phosphate buffer leaf extract, as well as the soaked ethanolic and aqueous leaf extracts, had the highest catalase activity, according to the study. These findings suggest that both the leaf and root of *Petiveria alliceae* may be good sources of natural antioxidants and might be useful in the treatment of diseases associated with oxidative stress. These predictors, however, need further work to validate reliability.

Keywords: Ultraviolet-visible; catalase; ethanolic; leaf; root.

1. INTRODUCTION

Free radicals like superoxide anion radicals (O^{2}-), hydroxyl radicals (OH^{-}), and non-free radical species like hydrogen peroxide (H_2O_2) and singlet oxygen are all examples of reactive oxygen species (ROS), which can harm cell structures like carbohydrates, nucleic acids, lipids, and proteins and change how they function [1-3]. Additionally, oxidative stress, a factor in numerous pathological conditions such as cancer, neurological disorders, atherosclerosis, hypertension, ischemia/perfusion, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and asthma, may be too much for the antioxidant to handle [4-6].

Harmful ROS in animals and humans can be scavenged by phytochemical compounds, such as alkaloids and flavonoids which are antioxidants that have potential health effects [7,8]. For decades, modern drugs have been isolated from medicinal plants, and recently much attention has been dedicated to antioxidants and their association with health benefits [9,10]. The presence of phytochemical constituents in plants is necessary for their biological activities [11] and these can be derived from the different parts of the plant such as the leaf, stem, seed, fruit and root that work with the nutrients and fibers to act as a defense system or to protect against disease [12].

Petiveria alliacea is a medicinal plant belonging to the Phytolaccacea family. It is a perennial shrub that has been used for centuries as an analgesic, anti-inflammatory, anti-microbial, anti-cancer, anti-rheumatic, antispasmodic, diuretic, sedative, abortive, and hypoglycemic agent [13-16]. To establish its medicinal effect, the study was designed to assess the phytochemical profiles and evaluate the in vitro antioxidant capacities of aqueous and ethanolic leaf and root extracts of *Petiveria alliacea*.

2. METHODOLOGY

2.1 Collection of Plant Materials and Extraction Procedures

Petiveria alliacea leaves and roots were collected from a farm at Ado-Ota Local Government Area, Ogun State, Nigeria, and authenticated in the Department of Botany, Lagos State University, Ojo, Nigeria. The leaves and roots were cleaned in running water to remove impurities and air-dried under ambient conditions (30 ± 2^{0}C) for seven (7) days. The dried samples were ground into a fine powder with a high speed blender (Kenwood BLK - 828), then stored in an airtight container at room temperature. The aqueous and ethanolic leaf and root extraction was carried out according to the modified method of Ogunrinola et al. [17]. Briefly, 200 g of *Petiveria alliacea* leaves and roots were boiled with 500 ml of water, and refluxed in ethanol, respectively, for 10 minutes. The resulting solutions were then soaked for 48 hours in 500 mL of water and ethanol, respectively. The obtained extracts were filtered over Whatman No. 1 paper, and the filtrate was concentrated by a rotary evaporator at 50°C and kept for further analysis.

2.2 Phytochemical Profile

2.2.1 Qualitative analysis for phytochemical constituents

The following phytoconstituents: tannins, phlobatannins, saponins, flavonoids, terpenoids, cardiac glycosides, phenolics, alkaloids in the aqueous and ethanolic extracts of *Petivera alliacea* leaves and roots respectively using modified standard procedures described by Kalaichelvi and Dhivya [18], Edeoga et al. [19], Ayoola et al. [20], Trease and Evans [21], Ogunrinola et al. [17] and Harborne, [22].

2.2.2 Quantitative analysis for phytochemical constituents

The amounts of alkaloids and flavonoids present in the crude powdered samples of *Petivera alliacea* leaves and roots were evaluated using standard procedures based on the modified methods of Ayodele et al. [23], Boham et al. [24] and Harborne [22].

2.3 Ultraviolet-visible Spectroscopic Analysis

The qualitative analysis for the phytochemical constituents of aqueous and ethanolic *Petivera alliacea* leaf and root extracts by UV spectrophotometry were carried out by the modified methods of Karpagasundari and Kulothungan [25], Janakiraman et al. [26], and Hussain et al. [27]. Briefly, the soaked and unsoaked extracts were centrifuged at 3000 rpm for 10 minutes and filtered through Whatman No. 1 filter paper. The samples were dissolved in 50 mM Tris-HCl buffer (pH 7.0), to prepare stock solution of concentration of 5 mg/ml. The working solutions (0.5 mg/ml) were prepared by diluting 1 ml of the stock solution with 10 ml 50 mM Tris-HCl buffer (pH 7.0) (1:10). Different concentrations (0.01, 0.025, 0.075, 0.1 and 0.15 g/ml) of the extracts were prepared with 50 mM Tris-HCl buffer (pH 7.0), and scanned at a wavelength ranging from 378-654 nm using Thermo Scientific Spectroscan Ultraviolet-Visible (UV) 2700 double beam Spectrophotometer, and the characteristic peaks were detected using Tris-HCL buffer as a blank. The UV peak values were recorded. For spectrum confirmation, each and every analysis was repeated twice.

2.4 Evaluation of *In Vitro* Antioxidant

2.4.1 Hydrogen peroxide scavenging effects

The scavenging activity of *Petivera alliacea* aqueous and ethanolic extracts of leaves and roots was evaluated by the modified method of Ruch et al. [28]. Briefly, a solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (0.1M, pH 7.4). Concentrations of *Petiveria alliacea* leaf and root extracts ranging from 0.1 - 0.6 mg/ml were added to the hydrogen peroxide buffer solution, mixed, and absorbance at 230 nm against the phosphate buffer as blank was taken after 10 minutes. The percentage of hydrogen peroxide (H_2O_2) inhibition was calculated as:

% Scavenging of H_2O_2 = [(AC - AS)/AC] x 100; where AC was the absorbance of the control, and AS was the absorbance of the extracts and standard.

2.4.2 Evaluation of catalase activity

The modified methods of Aebi [29] and Luck [30] were employed to assay for catalase activity of *Petiveria alliacea* leaf and root extracts. In brief, a 20% homogenate of *Petiveria alliacea* leaf and root extracts was prepared in phosphate buffer (0.067M, pH 7.0). 2 ml of homogenate was mixed with 1 ml of hydrogen peroxide solution (2mM) in a quart cuvette. Also, 2 ml of soaked (for 24 hours), and unsoaked aqueous and ethanolic extracts were mixed with 1 ml of hydrogen peroxide (2mM). Catalase activity was assayed by measuring hydrogen peroxide consumption at 240 nm for 180 seconds with a 30 second interval, as indicated by a decrease in absorbance. The control and blank were assay solution without the extract. Enzyme activity was expressed as catalase unit/g protein.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Qualitative analysis of ethanolic and aqueous leaf and root *Petiveria alliacea* extracts

Table 1 shows the phytochemical constituents of the ethanolic and aqueous *Petiveria alliacea* leaf and root extracts. Alkaloids, terpenoids, tannins, saponins, phlobatannins, flavonoids and cardiac glycosides were present in both extracts.

3.1.2 Quantitative analysis of ethanolic and aqueous leaf and root *Petiveria alliacea* extracts

The quantitative estimation of ethanolic and aqueous leaf and root *Petiveria alliacea* extracts is depicted in Table 2. While the ethanolic extract of *Petiveria alliacea* had a high level of alkaloids (38.3 w/w) in the leaf and flavonoids in the root (17.7 w/w), the aqueous leaves had a high level of flavonoids (28.3 w/w), but a low level of alkaloids and flavonoids in the root extracts.

3.1.3 Ultraviolet-visible Spectroscopic of ethanolic and aqueous leaf and root *Petiveria alliacea* extracts

The qualitative UV spectrophotometric profiles of different concentrations of aqueous and ethanolic extracts of *Petiveria alliacea* leaves and roots are

shown in Figs. 1-5. The absorption of 0.01 g/ml of aqueous and ethanolic extracts of *Petiveria alliacea* at different wavelengths is depicted in Figs. 1a and 1b. The leaves and root aqueous extracts showed a high absorptivity at 378 nm which further decreased as the wavelength increased. While the root ethanolic extract showed the same pattern, the leaf ethanolic extract had increased absorptivity at 378 nm, which decreased at a wavelength of 608 nm and increased again at a wavelength of 654 nm.

The UV absorption spectrophotometric profile of 0.025 g/ml aqueous and ethanolic extracts of *Petiveria alliacea* is shown in Figs. 2a and 2b. All the extracts showed the same pattern of decreasing intensity as the wavelength increased.

The UV absorption spectrophotometric profile of 0.075 g/ml aqueous and ethanolic extracts of *Petiveria alliacea* is depicted in Figs. 3a and 3b, in which the leaves and root aqueous extracts (Fig. 3a) showed a high absorptivity at wavelength 378 nm, which decreased as the wavelength increased. In Fig. 3b, there was an up-and-down absorptivity of the leaf and root ethanolic extracts.

Table 1. Qualitative analysis of ethanolic and aqueous leaf and root *Petiveria alliacea* extracts

Phytochemical constituents	Ethanolic extract		Aqueous extract	
	Leaves	Root	Leaves	Root
Tannins	+	+	+	+
Phlobatannins	+	+	+	+
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Terpenoids	+	+	+	+
Cardiac Glycosides	+	+	+	+

+ = positive

Table 2. Quantitative analysis of ethanolic and aqueous leaf and root *Petiveria alliacea* extracts

Phytochemical constituents	Ethanolic extract		Aqueous extract	
	Leaf (w/w)	Root (w/w)	Leaf (w/w)	Root (w/w)
Alkaloids	38.3	2.1	6.8	0.5
Flavonoids	5.3	17.7	28.3	0.3

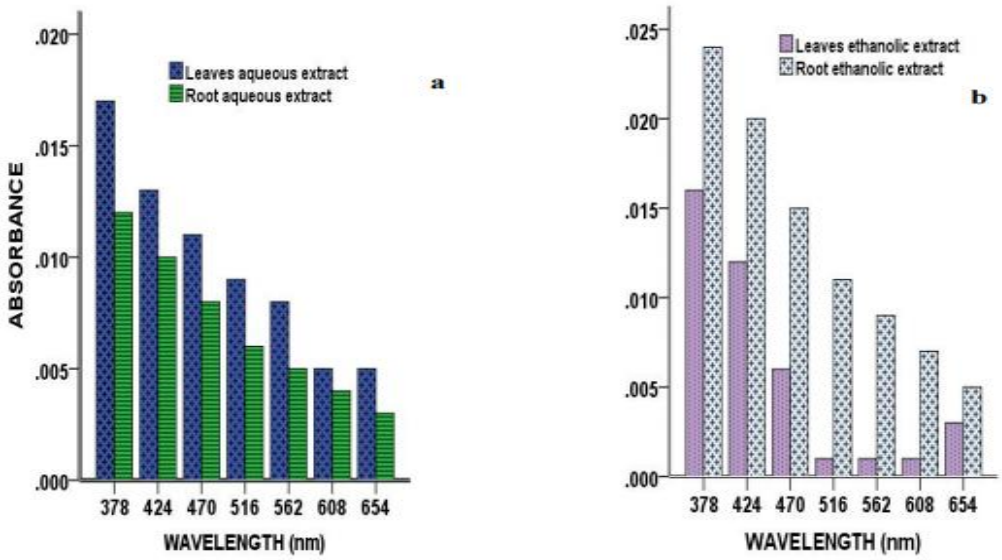

Fig. 1. UV-Visible spectrophotometric profile of 0.01g/ml aqueous (a), and ethanolic (b) extracts of *Petiveria alliacea* leaf and root

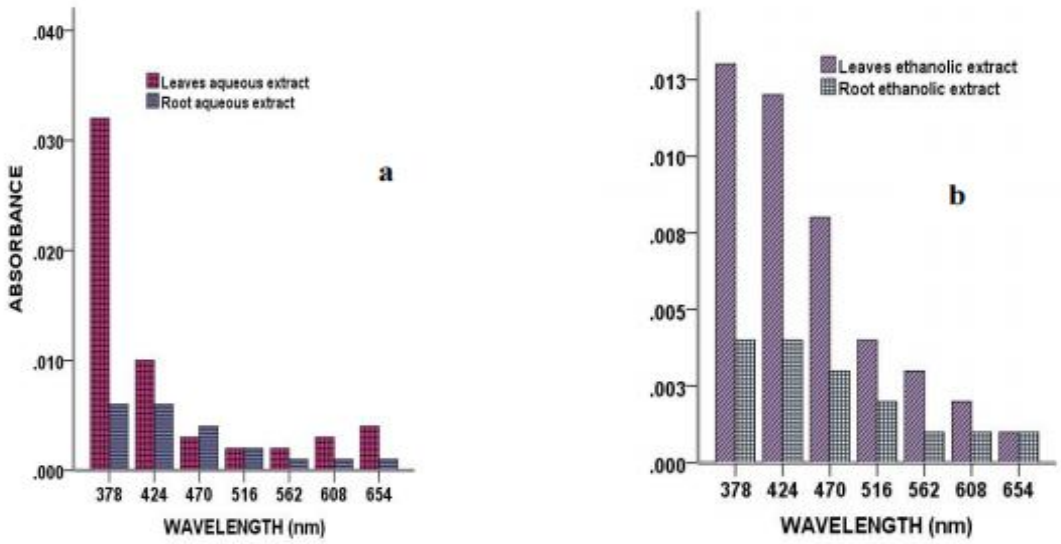

Fig. 2. UV-Visible spectrophotometric profile of 0.025g/ml aqueous (a), and ethanolic (b) extracts of *Petiveria alliacea* leaves and root

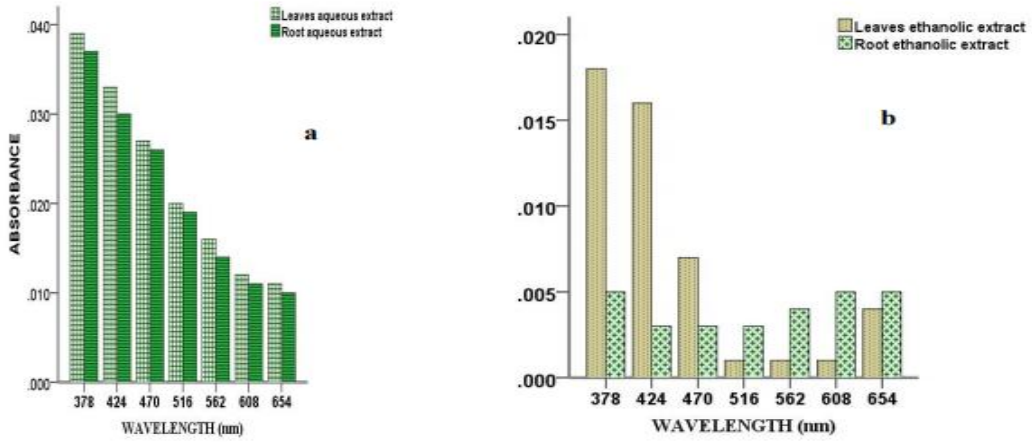

Fig. 3. UV-Visible spectrophotometric profile of 0.075g/ml aqueous (a), and ethanolic (b) extracts of *Petiveria alliacea* leaf and root

The UV absorption spectrophotometric profile of 0.1 g/ml aqueous and ethanolic extracts of *Petiveria alliacea* shown in Figs. 4a and 4b revealed a decrease in absorptivity as the wavelength increased for both leaves and roots aqueous extracts (Fig. 4a) and an up-and-down absorptivity of the leaves and roots ethanolic extracts (Fig. 4b).

Figs. 5a and 5b indicated that the absorptivity of the root aqueous extract was higher than that of the leaves, but both extracts followed the pattern of increased absorptivity as wavelength increased (Fig. 5a), while the ethanolic leaf extract (Fig. 5b) showed the highest absorptivity in an up-and-down manner.

3.1.4 Hydrogen peroxide scavenging effects of ethanolic and aqueous leaf and root *Petivera alliacea* extracts

As shown in Fig. 6, the aqueous extracts of *Petivera alliacea* demonstrated increased hydrogen peroxide decomposition activity of 10.4 % (leaves) and 11.7 %, 11.8 % (root), respectively, in a concentration dependent manner.

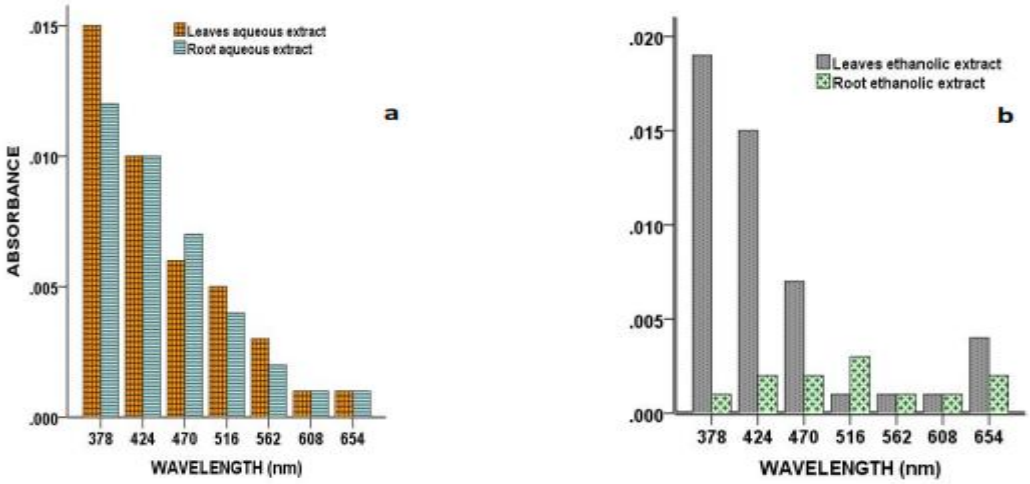

Fig. 4. UV-Visible spectrophotometric profile of 0.1g/ml aqueous (a), and ethanolic (b) extracts of *Petiveria alliacea* leaves and root

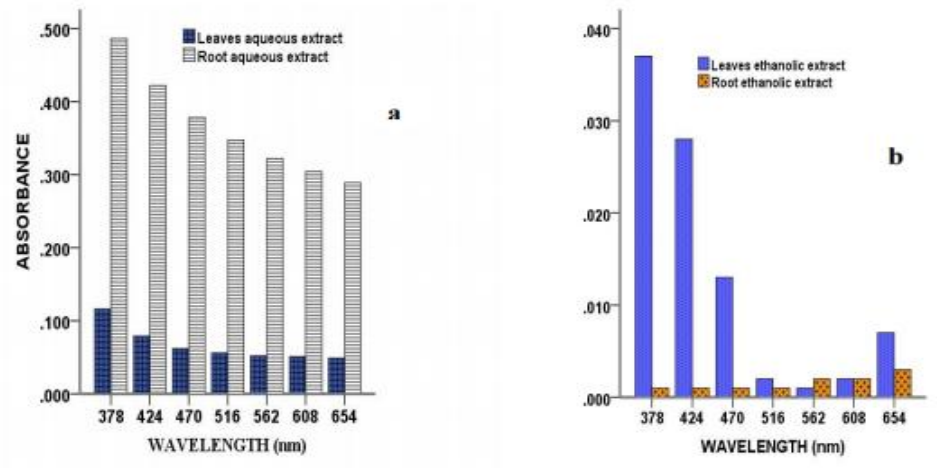

Fig. 5. UV-Visible spectrophotometric profile of 0.15g/ml aqueous (a), and ethanolic (b) extracts of *Petiveria alliacea* leaf and root

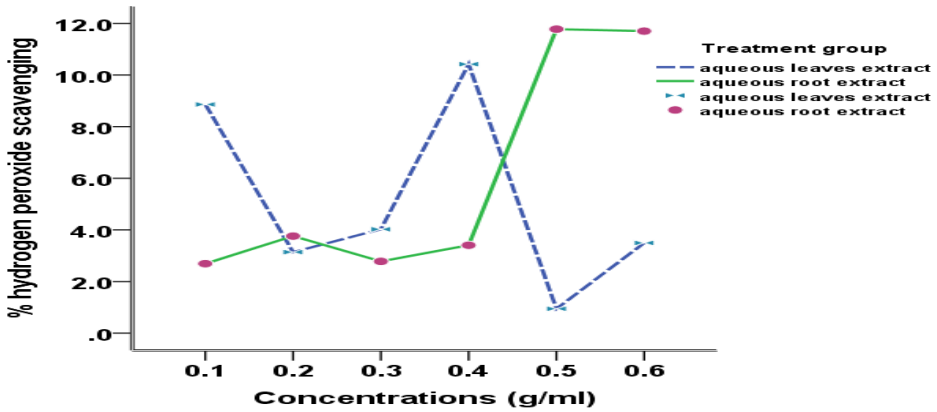

Fig. 6. The % hydrogen peroxide scavenging activity against different concentrations of aqueous root and leaf *Petiveria alliaceae* extracts

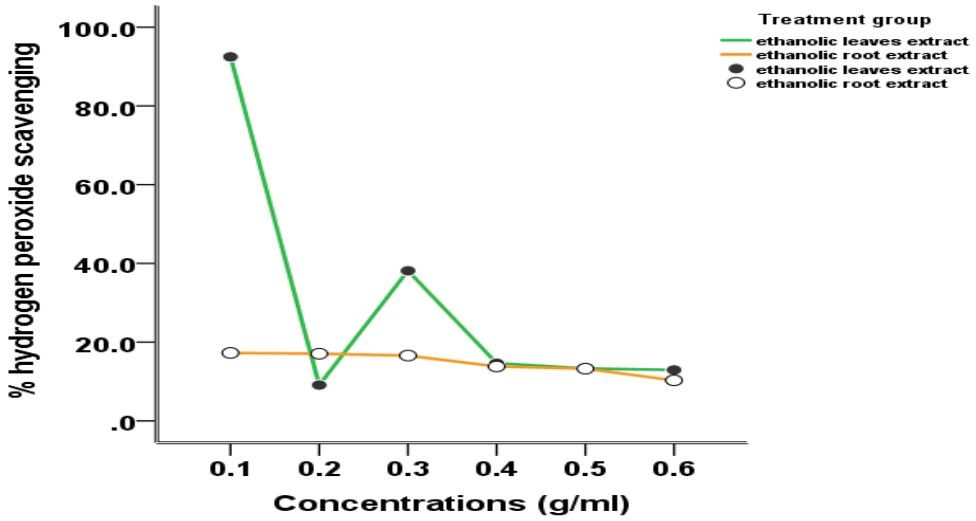

Fig. 7. The % hydrogen peroxide scavenging activity against different concentrations of ethanolic root and leaf *Petiveria alliaceae* extracts

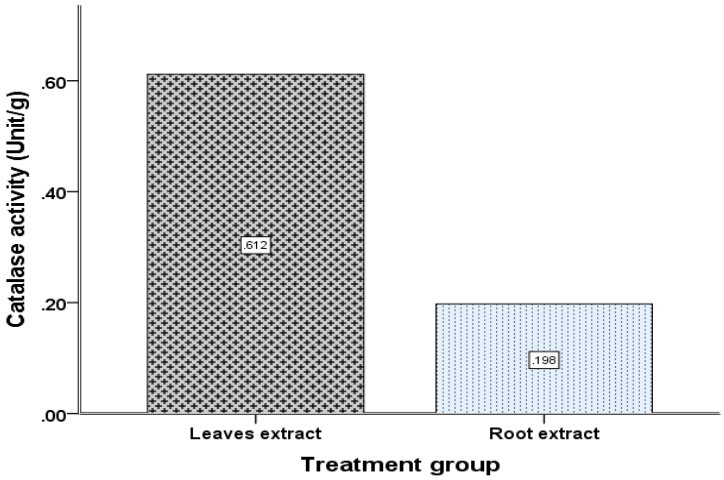

Fig. 8. Catalase activity (unit/g) of phosphate buffer *Petiveria alliacea* leaf and root extract

Fig. 7 shows that the ethanolic leaf extract had a high level of hydrogen peroxide decomposition at a 0.1 g/ml concentration compared to the root extract.

Phosphate buffered leaf extracts (Fig. 8) exhibited higher catalase activity (0.612 Unit/g) compared to the root extract (0.198 Unit/g). Fig. 9 depicts the catalase activity of soaked and unsoaked aqueous and ethanolic extracts of *Petiveria alliacea* leaves and root respectively. There was a high level of catalase activity in the ethanol-soaked leaf and root extracts, representing 0.198 Unit/g and 0.076 Unit/g, respectively, compared to the unsoaked extract. The aqueous soaked leaves extract had a high catalase activity of 0.102 Unit/g compared to the root extract, and the unsoaked aqueous root extract has a high activity (0.07 Unit/g) compared to the leaves extract.

3.2 Discussion

Screening for potential phytochemicals and antioxidant agents in natural plant products is still the main focus of many therapeutic scientists because antioxidants have the capacity to stop the chain reaction of oxidative species and the deleterious health hazards they pose to the body. The pharmacological activities of any medicinal plant are due to the presence of the secondary metabolites: flavonoids, glycosides, terpenoids, alkaloids, tannins, saponins, carbohydrates, glycosides, flavonoids, and steroids [7,8,19].

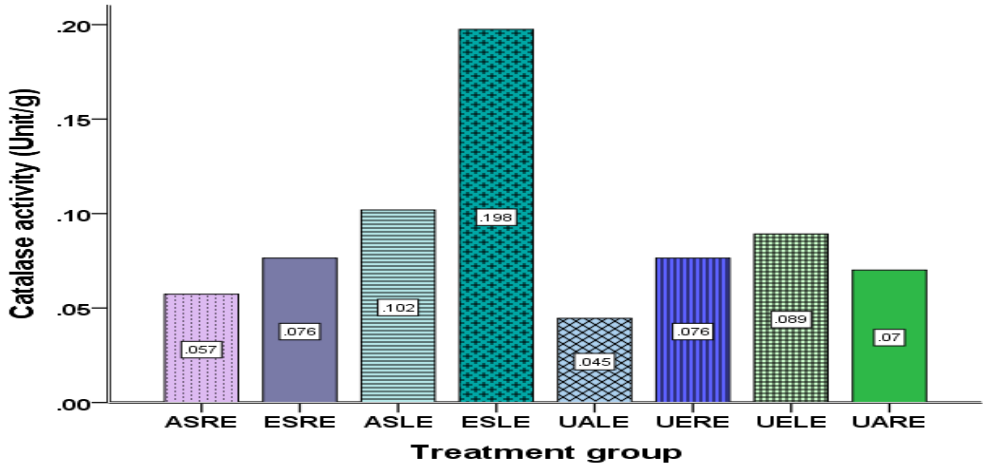

Fig. 9. Catalase activity (unit/g) of soaked and unsoaked aqueous and ethanolic extracts *Petiveria alliacea* leaf and root

ASRE = aqueous soaked root extract, ESRE = ethanolic soaked root extract, ASLE = aqueous soaked leaf exract, ELSE = ethanolic leaf soaked extract, UALE = unsoaked aqueous leaf extract, UERE = unsoaked ethanolic root extract, UELE = unsoaked ethanolic leaf extract, UARE = unsoaked aqueous root extracts

These metabolites have been reported as scavengers of ROS and capable therapeutic drugs for free radical mediated pathologies, including vascular diseases [31]. The present study shows that these metabolites are present in both the aqueous and ethanolic extracts of *Petiveria alliacea* leaves and roots, respectively. The results obtained are consistent with the findings of Sathiyabalan et al. [32] and Ayodele et al. [23]. The presence of these secondary metabolites explains the industrial and medicinal importance of the plant. This study observed the presence of high levels of alkaloids and flavonoids in ethanolic leaf, root, and aqueous leaf extracts of *Petiveria alliacea*.

Investigation of medicinal plants' compositions and activities are very important to promote their therapeutic purposes. The ultraviolet-visible spectroscopy profile of aqueous and ethanolic leaf and root *Petiveria alliacea* extracts of different concentrations revealed the highest peaks in the region from 378 to 470 nm. This is a clear indication of the presence of unsaturated groups and heteroatoms such as sulphur, nitrogen, and oxygen, which confirms the presence of organic chromophores such as phenolic, alkaloid, flavonoids, and glycosides compounds within the *Petiveria alliacea* extracts [33]. This is in accordance with the research of Syed and Johnson [34]; Mamta et al. [35]; and Neha and Jyoti [36].

Flavonoid and alkaloid compounds are known to exhibit biological activities including antipyretic, analgesic, anti-inflammatory, anti-arthritic, anti-carcinogenic, immunomodulatory and anti-atherosclerotic properties [37,38]. These activities might be related to their antioxidant activities [7], whereby they act in a structure-dependent manner; scavenge reactive oxygen species, and chelate transition metals, which play vital roles in the initiation of deleterious free radical reactions [39]. Antioxidants can be clarified as reductants, and in-activators of oxidants, whose reducing power may serve as a significant indicator of potential antioxidant activity [40]. Therefore, the *in vitro* antioxidant potential of different extracts of *Petiveria alliacea* leaves and roots was estimated. Hydrogen peroxide is a non-radical oxidizing specie formed in tissues by oxidative processes, which generate hydroxyl radicals and thus initiate and propagate lipid peroxidation [3]. The scavenging of hydroxyl radicals is a significant antioxidant activity for the protection of living systems from deleterious reactions between hydroxyl radicals and a wide range of molecules in living cells, like sugars, amino acids, lipids, and nucleotides [40]. The present study shows that the percentage hydrogen peroxide scavenging activity of aqueous *Petiveria alliacea* leaf and root extracts but not the ethanolic extracts increased in a concentration-dependent manner. This is consistent with the research of Sowndhararajan and Kang [40] and in the leaf as reported by Orogbodo et al. [41]. This implies that the aqueous extract will be able to scavenge hydroxyl radicals.

Catalase is a vital antioxidant enzyme that converts hydrogen peroxide into water and molecular oxygen using either an iron or manganese as cofactor, thereby protecting the tissues from hydroxyl radicals [42,43]. The study revealed the highest catalase activity in the phosphate buffer of *Petiveria alliacea* leaf extract and in the soaked and unsoaked leaves and root of *Petiveria alliacea* aqueous and ethanolic extracts. The ability of *Petiveria alliacea* extract to suppress oxidative stress *in vitro* through the antioxidant defense enzymes as observed in this study is similar to the previous reports of Ighodaro et al. [44] and Valyova et al. [45]. The presence of catalase, a potential scavenger of hydrogen peroxide, prevent the uncontrolled export of toxic species from organelles to the cytosol and compete to remove hydrogen peroxide. The phytoconstituents, especially alkaloids and flavonoids, found in *Petiveria alliacea* may be one of the reasons it can act as an antioxidant by releasing electrons to quiacol-type peroxidases for scavenging hydrogen peroxide, suppressing the formation of reactive oxygen species by inhibiting some enzymes or chelating trace metals involved in free radical production, and stimulating antioxidant defense [46,47].

4. CONCLUSION

The results obtained in the current study revealed that *Petiveria alliacea* leaves and roots are rich sources of phytoconstituents, which was confirmed by the spectroscopic profile. The antioxidant activity exhibited by the plant parts via free radical scavenging and catalase-reducing power might be attributed to its alkaloid and flavonoid contents. These findings therefore suggest that *Petiveria alliacea* leaves and roots might act as a source of natural antioxidants that could have enormous importance as therapeutic agents. However, additional research will be required to fully determine its bioactivity, and toxicity profile for proper use in the pharmaceutical industries.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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