

## **Evaluation of Phytochemical, Antimicrobial and Antioxidant Capacities of *Pennisetum purpureum* (Schumach) Extracts**

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### **Authors' contributions**

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

### **Article Information**

DOI: 10.9734/CSJI/2020/v29i430170

#### Editor(s):

(1) Dr. R. Rajalakshmi, Avinashlingam Institute for Women, India.

#### Reviewers:

(1) Maria Bintang, Bogor Agricultural University, Indonesia.

(2) Sandeep Onkar Waghulde, University of Mumbai, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/55961>

**Received 30 January 2020**

**Accepted 06 April 2020**

**Published 11 April 2020**

**Original Research Article**

### **ABSTRACT**

Medicinal plants are valuable natural sources for the development of potentially safe drugs. The biological activities associated with these plants are due to the presence of certain phytochemicals that act individually or synergistically. Thus, this study examined the phytochemical components, antioxidant and antimicrobial activities of n-hexane, ethylacetate and methanolic extracts of *Pennisetum purpureum* (Schumach). Qualitative and quantitative phytochemical assays of *P. purpureum* showed the presence of alkaloids (0.004%), saponins (0.002%), flavonoids (0.021%), steroids, terpenoids and glycosides (0.008%). Methanol, n-hexane and ethylacetate extracts of *P. purpureum* were examined for antimicrobial activity using the disc diffusion method. Six microbial strains were exposed to six different concentrations of each extracts; 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml. The three extracts demonstrated varied concentration-dependent antimicrobial activities against the test organisms. The methanolic extract showed antibacterial activity against *E. coli*, *S. aureus*, *B. cereus* and antifungal activity against *T. mentagrophyte* and *A. niger*. Among all extracts, the methanolic extract of *P. purpureum* exhibited relatively strong antifungal activity against *A. niger* (10.3±0.12 mm) when compared to the standard antifungal agent, fluconazole (13.9±0.12 mm). Furthermore, antioxidant activities were spectrophotometrically studied using vitamin C as standard; methanol and ethylacetate extracts

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of *P. purpureum* showed pronounced scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH); and had a potent reductive ability on ferric ion and phosphomolybdate. However, only the non-polar extract of *P. purpureum* showed a non-significant correlation and significant differences when compared to vitamin C. Antioxidant activities of the plant extracts were observed in the order of methanol >ethylacetate> n-hexane extracts. The results showed that *P. purpureum* contains phytochemicals that significantly contributed to the observed antimicrobial and antioxidant abilities of the plant and could be used as a potential source for the development of novel therapeutic drugs.

**Keywords:** Antimicrobial; antioxidant; constituents; dihydrocapsaicin; maceration; *Pennisetum purpureum*; phytochemical; sitosterol.

## 1. INTRODUCTION

The quest for plants with medicinal properties continues to receive attention for a wide range of biological activities. Within the last two decades, a large and ever-expanding global population prefers the use of natural products in treating and preventing medical problems [1]. In Nigeria, most communities use medicinal herbs to treat various diseases and ailments including asthma, tuberculosis, ulcers, diarrhea, dysentery, to name but a few [2]. Plants have been known to contain or possess abundant phytochemicals, antimicrobials and pharmacologically active principles, which include anthraquinones, flavonoids, saponins, polyphenols, tannins, and alkaloids [3].

While orthodox medicine is generally accepted and preferred globally, the use of herbs and traditional medicines is often considered an equally acceptable alternative in many regions of the world [2]. Traditional medicine is commonly used in developing countries where the cost of orthodox medicine and access to medical care are not available to a part of the population [2].

According to the World Health Organization (WHO), 65-80% of the world's populations rely on traditional medicine to treat various diseases [4]. Some medicinal herbs having antioxidant effects can also reduce blood lipids. Several herbal preparations from different parts of plants (leaves, roots, barks and twigs) have become popular for the treatment of a variety of diseases such as diabetes mellitus, breast cancer, hypertension and atherosclerosis [5]. Similarly, this study was designed to investigate the phytochemicals, antioxidant potential and antimicrobial activities of *P. purpureum* found in southeastern Nigeria.

*Pennisetum purpureum* (Schumach) commonly known as napier grass is a tall grassy perennial plant in the Poaceae family [6,7,8]. Although native to subtropical Africa, the apical ends of the

stems of napier grass plants usually have whorled tender young leaves before flowering [7]. The two common varieties of plants present in Nigeria are those with purple stem and light green stem; these are known respectively in local Igbo dialects as 'achara Ibeku' and 'achara Ngwa' cultivars. The naturally sheathed tender young leaves of napier grass is cherished as a soup vegetable, especially amongst Ngwa and Umuahia-Ibeku clans of Igbo people of Abia State, Nigeria. The ethnic soup made with this vegetable is called "ofe achara" [9]. The Igbo people consume this soup with complimentary ethnic viscous starchy pastes such as yam 'fufu' and 'eba' [10,11].

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

Fresh young shoots of *P. purpureum* were purchased from a local market in Port Harcourt, Nigeria. The fresh shoots were identified by Prof. (Mrs.) O. B. Green of the Department of Plant Science and Biotechnology, Rivers State University, Nigeria. The fresh shoots were washed with distilled water (without squeezing) to remove debris and dust particles. They were air-dried for a few days under shade to prevent ultra-violet rays from altering the chemical constituents [12]. The dry plant material was later pulverized using a manual blender.

### 2.2 Extraction

The dried pulverized sample of *P. purpureum* (780 g) was macerated using 3.95 L of n-hexane in an aspiratory bottle at room temperature for 48 hours with frequent stirring [13]. Then the extract obtained was filtered into a conical flask using a funnel and a filter paper to obtain the n-hexane extract. The residue left was again subjected to second successive extraction with n-hexane according to the procedure described above to obtain the second extract of n-hexane, this process was done six (6) times to exhaustively

extract the plant components. The same procedure was performed on the plant residue using 2.8 L of ethylacetate and 2.4 L of methanol sequentially. The three extracts obtained were then separately concentrated using a rotary evaporator at 45°C. The concentrated extracts were later weighed to obtain the yields and percentage yield for each extract was calculated.

### 2.3 Phytochemical Screening

Phytochemical examination was carried out on each of the extracts of *P. purpureum* using standard methods. Each of the concentrated extract was subjected to qualitative tests via standard procedures [14,15] to detect the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, steroids and cardiac glycosides.

### 2.4 Phytochemical Quantification

The methods of Wenkam [16] and Wills [17] were adopted for the preparation and extraction of *P. purpureum* for gas chromatography-mass spectroscopy (GC-MS) analysis.

### 2.5 Antimicrobial Assay

#### 2.5.1 Test microorganisms

Clinical isolates of two Gram-positive pathogenic bacteria (*Bacillus cereus* and *Staphylococcus aureus*), two Gram-negative pathogenic bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and two pathogenic fungi (*Aspergillus niger* and *Trichophyton mentagrophyte*) were used for this study. The microbes were obtained from University of Port Harcourt teaching hospital, Nigeria.

#### 2.5.2 Disc diffusion assay

Disc diffusion method of susceptibility testing, as described by Ndukwe et al. [18] and Mahmodi et al. [19] with some modifications was used to evaluate the antimicrobial properties of the extracts. In this method, broth cultures of microorganisms were first prepared by inoculating a colony or colonial material of each microorganism separately, into 10 ml sterile broth medium; nutrient broth for the bacteria, and potato dextrose broth (PDB) for the fungi. Inoculated nutrient broth tubes were incubated at 37°C for 6 hours to obtain a turbidity equivalent to 0.5 McFarland standards, while inoculated PDB broth tubes were incubated at 25°C for 24 hours. After incubation, the broth cultures of the bacteria were swab plated, separately, unto

sterile nutrient agar plates. This was also done for the fungi; however, Sabouraud dextrose agar (SDA) plates were used in place of nutrient agar plates. The number of agar plates used corresponded to the number of dilutions of the investigated extracts, the number of microorganisms subjected to the testing, and the number of standard drugs (positive controls) used.

Filter paper discs of 6 mm in diameter were impregnated separately with the *P. purpureum* extracts and placed on the inoculated plates with the aid of sterile forceps. Antibiotic and antifungal impregnated discs were also placed on another set of inoculated plates to serve as positive controls. The plates were incubated at 37°C for 24-48 hours, after which the zones of inhibition around the discs were measured.

### 2.6 Antioxidant Assays

#### 2.6.1 DPPH radical scavenging assay

The DPPH radical scavenging activity was determined according to the method reported by Sunil and Ignacimuthu [20] with few modifications. 1 ml of methanolic DPPH (0.15%) was mixed with 3 ml of each extract of *P. purpureum* at varying concentrations (0.25-2.5 mg/L) or vitamin C (reference antioxidant) and incubated in a dark room for 30 minutes. Thereafter, absorbance was measured at 515 nm. Scavenging activity of each extract and vitamin C was expressed as percentage and calculated using equation 1. Distilled water was used as blank

$$\% \text{ Scavenging Activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\% \quad (1)$$

Where,  $A_{\text{control}}$  is absorbance of control,  $A_{\text{sample}}$  is absorbance of sample.

#### 2.6.2 Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was investigated using the method described by Bera et al. [21] with few modifications. 1 ml of phosphate buffer (0.2 M, pH 7.2), 1 ml of test solution either *P. purpureum* extract (0.25-2.5 mg/L) or vitamin C, 0.02 ml of ferric chloride (0.02 M) and 0.05 ml of phenanthroline (0.04 M) were introduced into a test tube. The reaction was triggered by adding 0.05 ml of 7 mM hydrogen peroxide. After 5 minutes of incubation at room temperature (25°C), absorbance was measured at 560 nm using a UV spectrophotometer. Hydroxyl radical scavenging

activity was expressed as percentage scavenging activity and calculated using equation 2. Methanol was used as blank

$$\% \text{ scavenging Activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\% \quad (2)$$

Where,  $A_{\text{control}}$  is absorbance of control,  $A_{\text{sample}}$  is absorbance of sample.

### 2.6.3 Phosphomolybdate assay

Free radical scavenging activity via the phosphomolybdate method was determined according to the method of Jayaprakash et al. [22] as modified by Okoko and Diepreye [23]. 0.2 ml of either *P. purpureum* extract (0.25-2.5 mg/L) or vitamin C (reference antioxidant) was mixed with 1 ml of phosphomolybdate reagent (4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulphuric acid) and incubated in a water bath at 95°C for 90 minutes. Absorbance was taken at 695 nm after allowing content to cool. Free radical scavenging activity was expressed as percentage activity and calculated using equation 3. Distilled water was used as blank

$$\text{Total antioxidant capacity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\% \quad (3)$$

Where,  $A_{\text{control}}$  is absorbance of control,  $A_{\text{sample}}$  is absorbance of sample.

### 2.6.4 Reducing ability

The ability of the extract to reduce  $\text{Fe}^{3+}$  was investigated according to Oyaizu [24] method as modified by Okoko and Diepreye [23]. *P. purpureum* extract or vitamin C (0.5 ml) was mixed with 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of potassium ferricyanide (1%) and incubated at 50°C. After incubation for 20 minutes, 0.5 ml of trichloroacetic acid (10%) was added and centrifuged for 10 minutes at 3000 rpm. A portion of the upper layer (0.5 ml) was mixed with 0.5 ml distilled water and 0.1 ml ferric chloride (0.1%). After 10 minutes of incubation at room temperature, absorbance was measured at 700 nm. An increase in absorbance indicated greater reducing ability.

## 2.7 Statistical Analysis

Data obtained were expressed as mean  $\pm$  standard deviation (SD) of triplicates. All data were subjected to one-way analysis of variance (ANOVA) using SPSS (version 20) software. The values were considered to be significantly different when  $p < 0.01$ . Means and standard

deviations from the DPPH radical scavenging activity, hydroxyl radical inhibitory, reducing ability and phosphomolybdate assays are results of experiments performed in triplicate.

## 3. RESULTS AND DISCUSSION

Studies have shown that medicinal plants are excellent sources of antioxidants and are reported to play an important role in the treatment of diseases around the world [25,26]. In this study, extracts obtained from *P. purpureum* using maceration method were used for phytochemical screening (qualitative and quantitative), antimicrobial and antioxidant assays. The extraction process was carried out starting with a non-polar solvent (n-hexane), followed by a semi-polar solvent (ethylacetate) and finally a polar solvent (methanol). Percentage yields of *P. purpureum* extracts are shown in Table 1. Percentage of extractable compounds varied from 2.2% to 3.1%. This observation agrees with the work of Ibrahim et al. [27] who reported the percentage yield of extracts to be in the order, methanol > ethylacetate > n-hexane. However, the yield of the extracts, as well as the bioactivity of the extracts prepared via maceration extraction method has been reported to vary in several studies [28]. It has also been suggested that maceration method may be a better choice for extraction of secondary metabolites [28].

Qualitative phytochemical screening of the extracts of *P. purpureum* showed the presence of secondary metabolites (Table 2). Both methanolic and ethylacetate extracts revealed the presence of key secondary metabolites (alkaloids, saponins, flavonoids, steroids, terpenoids and cardiac glycosides) except tannins, while the n-hexane extract revealed the presence of steroids and terpenoids. Observed phytochemicals of *P. purpureum* corroborates the findings of Okaraonye & Ikweuchi [9] who reported the presence of saponins, alkaloids and flavonoids in the shoots of *P. purpureum*. Alkaloids have important pharmacological uses such as analgesics, antibacterial, antimalarial and anti-hypertensive [29]. Flavonoids are hydroxylated phenolic compounds and are reported to show antimicrobial [30], antiallergic, anticancer and antithrombotic activities [31]; they are also found to show effective antioxidant properties [30]. Saponins are reported to show immune-stimulating and anti-inflammatory activities [32] while steroids have been reported to possessed antimicrobial activities [33].

Gas chromatography coupled with mass spectrometry (GC-MS) is preferred for more precise information in both qualitative analysis and quantitative determination [34]. This study also includes a quantitative estimate of the percentage of alkaloids, glycosides, saponins and flavonoids components (Table 3). Flavonoids (0.021%) had the highest phytochemical presence in *P. purpureum* followed by glycosides (0.008%), with saponins (0.002%) as the lowest. The medicinal value of *P. purpureum* may be related to their constituent phytochemicals. According to Varadarajan et al. [35], secondary metabolites and other chemical constituents of medicinal plants account for their medicinal value. Some identified bioactive phytochemical constituents of the methanolic extract of *P. purpureum* (Table 4 and Fig. 1) are dihydrocapsaicin (1), ethyl iso-allocholate (2), 2-hydroxy-4-methoxybenzaldehyde (3), 1,2-benzenediol (4), ergost-5-en-3-ol (5) and stigmast-5-en-3 $\beta$ -ol (6). Dihydrocapsaicin (1) have been reported to have antioxidant activity [36,37]; ethyl iso-allocholate (2), an alkaloid has anti-inflammatory and antimicrobial properties [38]; ergost-5-en-3-ol (5) also known as campesterol is a cholesterol absorption reducing agent which possess antioxidant and anti-cancer properties [39]. Stigmast-5-en-3 $\beta$ -ol (6) also known as  $\beta$ -sitosterol is a phytosterol having anti-inflammatory, antipyretic, antiarthritic, anti-ulcer, insulin releasing and estrogenic effects. It is mainly known and used for its cholesterol lowering properties [40].

Extracts from *P. purpureum* demonstrated varied concentration-dependent antimicrobial activities against the test organisms. All the three extracts were active against *E. coli*, *S. aureus*, *B. cereus*, *T. mentagrophyte* and *A. niger* (Tables 5-7). However, *P. aeruginosa* was resistant to all three extracts at all concentrations. The ineffectiveness of these extracts against *P. aeruginosa* is not surprising since Gram-negative bacteria are more resistant than Gram-positive ones [41,42]. The reason for the differences in sensitivity between Gram-positive and Gram-negative bacteria could be attributed to the morphological difference between them [43,44,45]. Gram-negative bacteria have outer phospholipidic

membrane that carries structural lipopolysaccharide components making the cell walls impermeable to lipophilic solutes. Gram-positive bacteria, on the other hand, are more susceptible because they have only an outer peptidoglycan layer which is not an effective permeable barrier [45,46]. Therefore, the cell walls of Gram-negative organisms which are more complex than that of Gram-positive organisms act as a diffusional barrier making them less susceptible than Gram-positive bacteria to antimicrobial agents [45,46]. Results of *P. purpureum* indicate that methanol produced a more potent extract (with higher antimicrobial activity) which inhibited a greater number of the test bacterial and fungi strains. However, the activity of *P. purpureum* methanolic extract against *T. mentagrophyte* showed a relatively good effect when compared to the conventionally used standard antibiotic (fluconazole). Rahmoun et al. [47] and Vlachos et al. [48] reported similar findings on the high antibacterial activity of methanolic extract. Therefore, it can be said that the observed antimicrobial activity of the methanol extract from *P. purpureum* against certain bacterial strains and fungi could be as a result of polar compounds present in the plants. In addition, the antimicrobial activities displayed against *E. coli*, *S. aureus*, *B. cereus*, *T. mentagrophyte* and *A. niger* could be due to the synergistic effect of the bioactive compounds such as ethyl iso-allocholate (2) and 2-hydroxy-4-methoxybenzaldehyde (3) identified in the plant.

The observed antibacterial inactivity in some of the concentrations of the extracts is not surprising, as several plant extracts that have been found ineffective against certain test organisms at lower concentrations are attributed to the presence of lower amounts of antimicrobial compounds [47].

All *P. purpureum* extracts showed antifungal activity against tested fungal isolates. Most of the extracts inhibited the clinical isolate of *A. niger*; this can be attributed to the presence of phenolic compounds. The amphipathicity of these compounds may explain their interactions with bio-membranes causing the inhibitory effect [49].

**Table 1. Extraction yield of *P. purpureum***

Extract	Weight of extract (g)	Percentage yield (%)
n-Hexane	17.01	2.20
Ethylacetate	21.40	2.70
Methanol	24.23	3.10

**Table 2. Phytochemical groups present in *P. purpureum***

Phytochemical group	n-Hexane extract	Ethylacetate extract	Methanol extract
Alkaloids	-	+	+
Saponins	-	+	+
Tannins	-	-	-
Flavonoids	-	+	+
Steroids	+	+	+
Terpenoids	+	+	+
Cardiac glycosides	-	+	+

KEY: + Present; - Absent

**Table 3. Phytochemical percentage composition of *P. purpureum***

Phytochemical group	Percentage composition (%)
Alkaloids	0.004
Glycosides	0.008
Saponins	0.002
Flavonoids	0.021
Tannins	Nil
Total	0.035

**Table 4. Identified components of *P. purpureum* methanolic extract**

Compound	Molecular formula	Molecular weight (g mol <sup>-1</sup> )	Retention time (min)	Peak area (%)
1,2-Dihydroxybenzene (4)	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	110	10.25	0.37
Ethyl iso-allocholate (2)	C <sub>26</sub> H <sub>44</sub> O <sub>5</sub>	436	11.243	0.21
Ergost-5-en-3-ol (5)	C <sub>28</sub> H <sub>48</sub> O	400	12.15	0.40
4-Hydroxy-3,5,5-trimethyl-4-(3-oxo-1-1-butenyl)-2-cyclohexen-1-one	C <sub>13</sub> H <sub>18</sub> O <sub>3</sub>	222	12.210	0.05
Stigmast-5-en-3β-ol (β-Sitosterol) (6)	C <sub>29</sub> H <sub>50</sub> O	415	14.210	0.16
Dihydrocapsaicin (1)	C <sub>17</sub> H <sub>29</sub> NO <sub>3</sub>	307	16.771	0.14
5-Butylpyridine-2-carboxylic acid	C <sub>10</sub> H <sub>13</sub> NO <sub>2</sub>	195	19.873	0.31
2,3-Di-O-phenylboranediyl-alpha-d-mannofuranose	C <sub>18</sub> H <sub>18</sub> B <sub>2</sub> O <sub>6</sub>	260	21.202	3.87
2-Hydroxy-4-methoxybenzaldehyde (3)	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>	152	21.221	0.67
2,4a,5,8a-Tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-ol	C <sub>14</sub> H <sub>24</sub> O	204	21.499	0.18
Cholest-4-en-3-one	C <sub>27</sub> H <sub>44</sub> O	384	22.063	1.70
Ergosta-4,22-dien-3-one	C <sub>28</sub> H <sub>44</sub> O	397	22.360	1.26
Stigmast-4-en-3-one	C <sub>29</sub> H <sub>48</sub> O	412	22.977	1.84
Cholest-4-ene-3,6-dione	C <sub>27</sub> H <sub>42</sub> O <sub>2</sub>	399	24.922	0.53
N-methyl-adamantane acetamide	C <sub>13</sub> H <sub>21</sub> NO	207	30.247	0.05
3,5-Bis(1,1-dimethylethyl)-4-hydroxy-2-4-cyclohexadien-1-one	C <sub>14</sub> H <sub>22</sub> O <sub>2</sub>	284	31.352	0.07

It has been suggested that extract components cross the cell membrane, interacting with enzymes and proteins of the membrane, thus producing a flux of protons towards the cell exterior which induces changes in the cells and eventually leads to their death [50]. It is evident from the results of this study that susceptibility of pathogens to plant extracts depends on the solvent used for extraction, the concentration of the extract and the organism tested, as

demonstrated in many studies [51,52]. This is in line with the results of a study reporting n-hexane to be less effective than methanol for the extraction of active plant compounds [53].

Among the standard drugs used, streptomycin had the highest zone of inhibition against *S. aureus* while fluconazole showed the lowest zone of inhibition against *T. mentagrophyte*.

### 3.1 DPPH Radical Scavenging Activity

Comparable scavenging activities of *P. purpureum* extracts with that of the standard (vitamin C) are given in Table 8. The results revealed significant difference with n-hexane extract while for ethylacetate and methanolic extracts, there were no significant difference. It was also observed that the scavenging effect of *P. purpureum* extracts on DPPH radical was in the order, methanol >ethylacetate> n-hexane extracts. The scavenging capacity of *P. purpureum* extracts may be due to the presence of phenolic compounds such as 2-hydroxy-4-methoxybenzaldehyde (3) and 1,2-dihydroxybenzene (4). Phenolic contents of plant extracts have been found to correlate with radical scavenging activity [54,55]. This is because, polyphenolics have high redox potentials that allow them to act as reducing agents, hydrogen

donors and singlet oxygen quenchers [56]. This might have played an important role in eradicating the radicals. It was observed that there exists a strong significant correlation ( $R^2 = 0.9711$ , Fig. 4) in the activity of methanolic extract of *P. purpureum* when compared to vitamin C. In contrast, n-hexane extract showed a non-significant correlation with a negative slope ( $R^2 = 0.0837$ ,  $R^2 = 0.9281$ ; Fig. 2) indicating that there is little or no scavenging activity in the n-hexane extract. The trend in the behavior of methanolic extract was similar to that of vitamin C. Fig. 3 depicts that the radical scavenging activity is in positive correlation with the concentrations of the ethylacetate extract used. However, correlation in the case of n-hexane was found to be non-significant (Fig. 2). This result is consistent with another report of a strong correlation of antioxidant activity in methanolic extract [57].

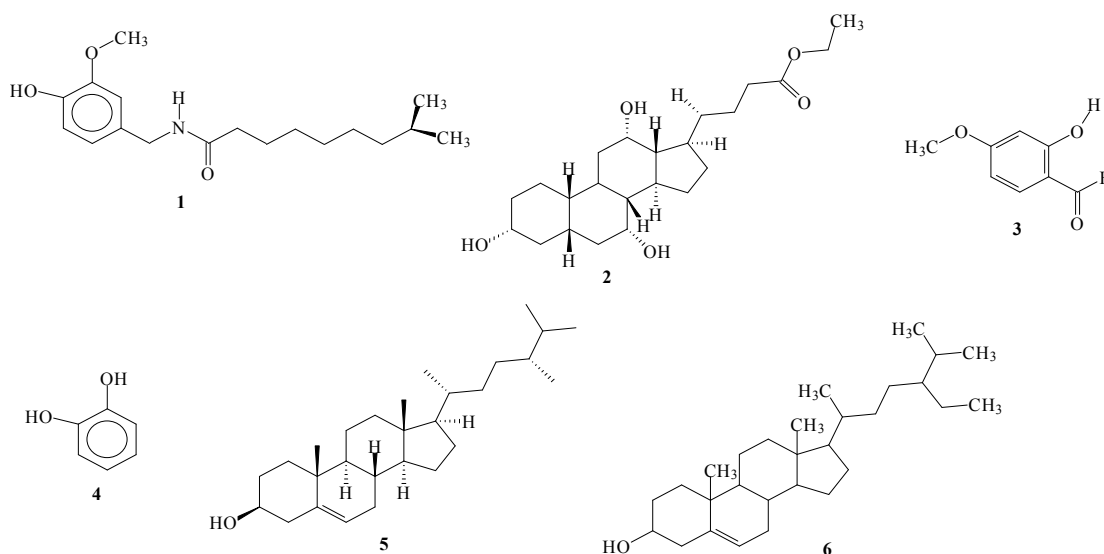


Fig. 1. Structures of some identified compounds of *P. purpureum* methanolic extract

Table 5. Antimicrobial activity of n-Hexane extract of *P. purpureum*

MCO	Zone of inhibition (mm)						
	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	PCT
S.a	9.9±0.10	9.0±0.06	8.3±0.27	7.4±0.29	NA	NA	28.9±1.05(ST)
B.c	10.5±0.55	9.5±0.23	8.1±0.69	NA	NA	NA	19.8±0.12(ST)
E.c	9.3±0.10	9.0±0.06	8.6±0.78	8.3±0.95	7.5±0.07	NA	19.3±0.35(OF)
P.a	NA	NA	NA	NA	NA	NA	15.9±0.21(OF)
A.n	9.0±0.15	8.5±0.12	7.8±0.12	7.2±0.06	NA	NA	13.9±0.12(FZ)
T.m	9.4±0.17	7.9±0.21	NA	NA	NA	NA	12.0±0.10(FZ)

Key: NA means no action, MCO – microorganisms, B. c – *Bacillus cereus*, S. a – *Staphylococcus aureus*, E. c – *Escherichia coli*, P. a – *Pseudomonas aeruginosa*, A. n – *Aspergillus niger*, T. m – *Trichophyton mentagrophyte*, PCT – positive control: ST- *Streptomycin*, OF – *Ofloxacin*, FZ – *Fluconazole*

**Table 6. Antimicrobial activity of ethylacetate extract of *P. purpureum***

MCO	Zone of inhibition (mm)						
	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	PCT
S.a	9.0±0.15	8.9±0.15	8.8±0.12	8.6±0.15	8.3±0.32	8.6±0.12	28.9±1.05(ST)
B.c	9.2±0.10	8.3±0.21	NA	NA	NA	NA	19.8±0.12(ST)
E.c	9.7±0.17	9.0±0.10	8.6±0.06	8.2±0.25	7.5±0.29	NA	19.3±0.35(OF)
P.a	NA	NA	NA	NA	NA	NA	15.9±0.21(OF)
A.n	9.9±0.15	9.1±0.06	8.7±0.12	8.1±0.29	7.9±0.45	7.8±0.32	13.9±0.12(FZ)
T.m	13.0±0.58	9.1±0.78	8.8±0.72	NA	NA	NA	12.0±0.10(FZ)

Key: NA means no action, MCO – microorganisms, B. c – *Bacillus cereus*, S. a – *Staphylococcus aureus*, E. c – *Escherichia coli*, P. a – *Pseudomonas aeruginosa*, A. n *Aspergillus niger*, T. m – *Trichophyton mentagrophyte*, PCT – positive control: ST- Streptomycin, OF – Ofloxacin, FZ – Fluconazole

**Table 7. Antimicrobial activity of methanol extract of *P. purpureum***

MCO	Zone of inhibition (mm)						
	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	PCT
S.a	12.4±1.25	9.8±0.81	9.3±0.21	8.7±0.36	7.9±0.21	7.6±0.38	28.9±1.05(ST)
B.c	10.2±0.90	8.9±0.15	8.5±0.15	8.4±0.10	8.0±0.21	7.7±0.31	19.8±0.12(ST)
E.c	10.5±0.10	9.5±0.15	8.7±0.12	8.4±0.06	8.6±0.25	8.3±0.25	19.3±0.35(OF)
P.a	NA	NA	NA	NA	NA	NA	15.9±0.21(OF)
A.n	10.3±0.12	9.5±0.17	8.8±0.15	8.6±0.27	8.3±0.31	7.8±0.44	13.9±0.12(FZ)
T.m	9.3±0.15	8.9±0.10	8.6±0.10	8.4±0.15	8.2±0.21	8.0±0.27	12.0±0.10(FZ)

Key: NA means no action, MCO – microorganisms, B. c – *Bacillus cereus*, S. a – *Staphylococcus aureus*, E. c – *Escherichia coli*, P. a – *Pseudomonas aeruginosa*, A. n *Aspergillus niger*, T. m – *Trichophyton mentagrophyte*, PCT – positive control: ST- Streptomycin, OF – Ofloxacin, FZ – Fluconazole

**Table 8. ANOVA result for antioxidant activity of *P. purpureum* extracts**

Extract	Methods			
	HRIA (560 nm)	DPPH (515 nm)	Phosphomolybdate (695 nm)	Reducing ability (700 nm)
Vitamin C	0.41±0.15	0.54±0.14	0.45±0.04	0.33±0.07
Methanol extract	0.41±0.09	0.51±0.09	0.46±0.06	0.30±0.11
n-Hexane extract	0.30±0.08*	0.41±0.12*	0.40±0.03	0.27±0.06*
Ethylacetate extract	0.34±0.10*	0.48±0.07	0.40±0.05	0.30±0.06
P-value	0.0206	0.1930	0.0925	0.5436
F-value	3.477	1.619	1.569	0.7232
Summary	S	Ns	Ns	Ns

Summary of the six varying concentrations (0.25, 0.50, 1, 1.5, 2.0 and 2.5 mg/L) of different solvent extract of *P. purpureum*. Values are the mean ± SD of samples in triplicate. Means present a significant difference ( $p < 0.01$ ).

\*Significance difference versus vitamin C, S-significant, NS – No significant difference

### 3.2 Reducing Power

The reducing power assay is often used to assess the ability of an antioxidant to donate an electron [24]. In this study, the ability of *P. purpureum* extracts was estimated in order to determine the potential of the extracts in reducing  $Fe^{3+}$  by electron donation to  $Fe^{2+}$ . This ability is associated with the presence of a redundant or complex molecule serving as an electron donor and/or free radical scavenger.

Vitamin C demonstrated much higher antioxidant activity than the n-hexane extract. In comparison, the ethylacetate and methanolic extract of *P. purpureum* had better ferric reducing ability than n-hexane extract (Table 8). This could be as a result of the phenolic compound (1,2-dihydroxybenzene) present in *P. purpureum* (Table 4, Fig. 1). 1,2-Dihydroxybenzene (4) also known as catechol, has been reported to possess anticancer, analgesic and antioxidant properties [58]. The reducing power of *P.*



*purpureum* extracts was found to be in the order, methanol > ethylacetate > n-hexane; indicating that the methanolic extract has the ability to reduce oxidative stress. Methanolic extract of *P. purpureum* may, therefore, contain high amount of reductones compared to ethylacetate extract and most likely absent in n-hexane extract. Therefore, the methanolic extract can act as electron donor and could react with free radicals to convert them into more stable products and terminate the free radical chain reactions. This result confirms the findings of El-Hashasa et al. [59], who reported that the reducing power of a plant correlates with its phenolic content. Fig. 11 shows that the reducing ability is in positive correlation with the concentrations of the methanolic extract used. A significant positive correlation (Fig. 13) was observed between the ethylacetate extract and vitamin C. While the n-hexane extract exhibited a non-significant correlation (Fig. 12).

### 3.3 Hydroxyl Radical Inhibitory Activity

The hydroxyl radical is one of the most reactive oxygen species in living systems, which can react with all possible molecules in living organisms, especially proteins, DNA and lipids [60]. Thus, removing OH<sup>•</sup> radical is very important for the protection of biological systems. In this study, the results show that the methanolic extract of *P. purpureum* was more active than other extracts and was approximately equal to vitamin C (Table 8). Correlation coefficient of methanolic extract was  $R^2 = 0.6989$ , showing that the inhibitory activities of *P. purpureum* was slightly higher than that of vitamin C ( $R^2 = 0.6773$ , Fig. 7). The R-value of ethylacetate extract appears to be better than that of n-hexane extract (Figs. 5 and 6). This could be as a result of the polarity index of n-hexane (0.1). Polyphenols and alkaloids which are most unlikely to be present in n-hexane extracts, are major antioxidants in natural products and their antioxidant activities have been proven in recent studies [61,62]. The activity of extracts, especially methanol and ethylacetate extracts are indications of the presence of phytochemicals that could be responsible for the antioxidant activity in *P. purpureum*.

### 3.4 Phosphomolybdate Scavenging Activity

Phosphomolybdate test measures the ability of an extract to destroy free radicals by transferring an electron to the later. The antioxidants present

in the extract reduce molybdate (VI) to molybdate (V) and this can be measured spectrophotometrically at 695 nm [22]. It was observed (Table 8) that the antioxidant capacity of the extracts of *P. purpureum* is in the order, methanol > ethylacetate > n-hexane. However, the antioxidant activity of vitamin C, an antioxidant used as the positive control, showed no significant differences with that of the extracts of *P. purpureum*. This could be attributed to the presence of phenolic compounds (1, 2-dihydroxybenzene (4) and 2-hydroxy-4-methoxybenzaldehyde (3)) identified in *P. purpureum*. 1,2-Dihydroxybenzene (4) and 2-hydroxy-4-methoxybenzaldehyde (3) have both been reported to act as antioxidants [36,58]. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to phosphomolybdate scavenging activity of medicinal plants [63,64]. There was a strong linear relationship between the activity of ethylacetate and vitamin C (Fig. 8). In contrast, n-hexane exhibited no correlation or relationship between the two variables (Fig. 9). The differences in observed antioxidant properties (Figs. 8-10) may be due to the polarity of extraction solvents.

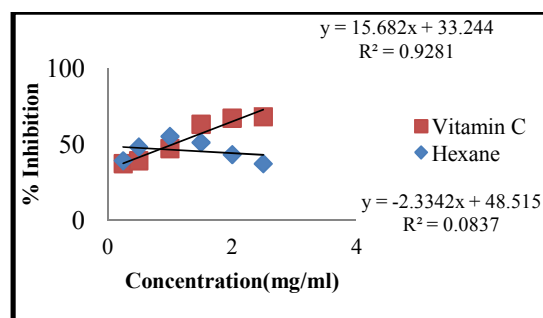


Fig. 2. DPPH free radical scavenging activity of n-hexane of extract of *P. purpureum*

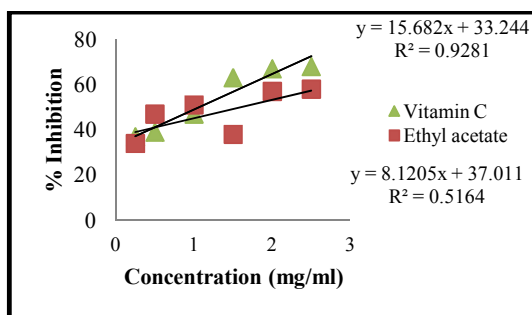


Fig. 3. DPPH free radical scavenging activity of ethylacetate extract of *P. purpureum*

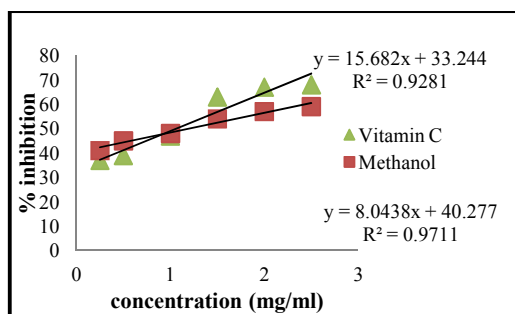


Fig. 4. DPPH free radical scavenging activity of methanol extract of *P. purpureum*

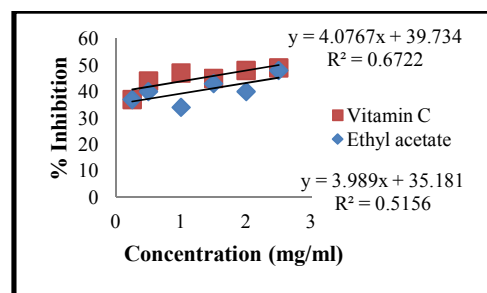


Fig. 8. Phosphomolybdate scavenging activity of ethylacetate extract of *P. purpureum*

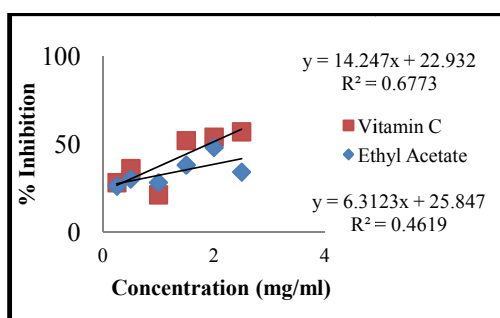


Fig. 5. Hydroxyl radical inhibitory activity of ethylacetate extract of *P. purpureum*

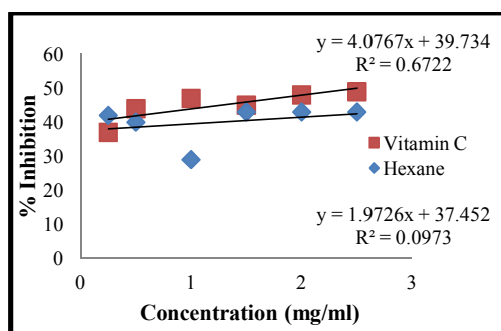


Fig. 9. Phosphomolybdate scavenging activity of n-hexane extract of *P. purpureum*

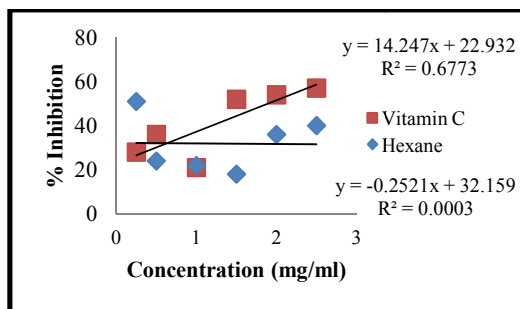


Fig. 6. Hydroxyl radical inhibitory activity of n-hexane extract of *P. purpureum*

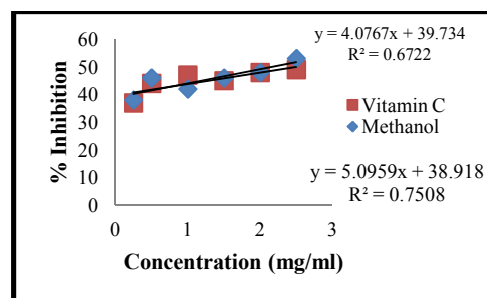


Fig. 10. Phosphomolybdate scavenging activity of methanol extract of *P. purpureum*

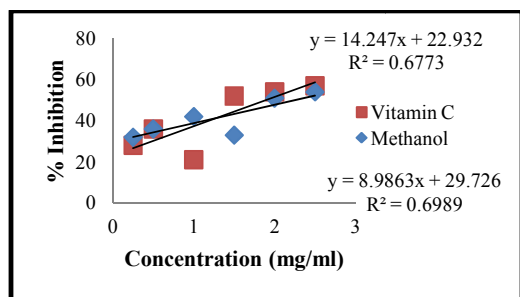


Fig. 7. Hydroxyl radical inhibitory activity of methanol extract of *P. purpureum*

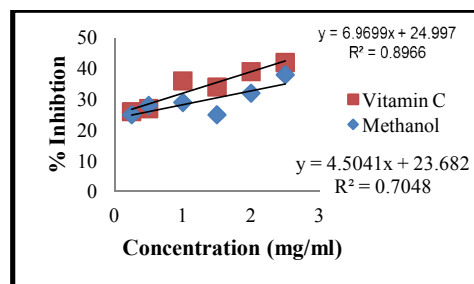


Fig. 11. Reducing ability of methanol extract of *P. purpureum*

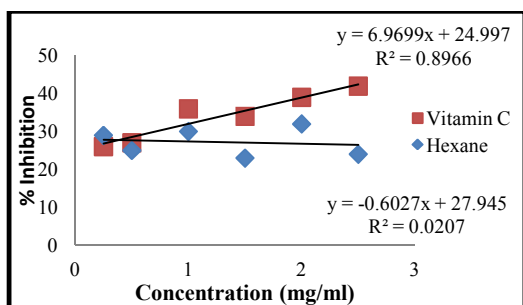


Fig. 12. Reducing ability of n-hexane extract of *P. purpureum*

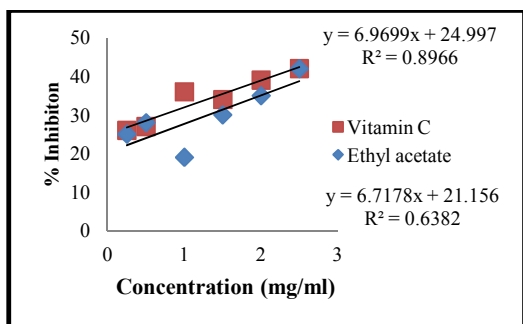


Fig. 13. Reducing ability of ethylacetate extract of *P. purpureum*

#### 4. CONCLUSION

*P. purpureum* showed strong antioxidant activities. It also exhibited moderate antimicrobial activities against some pathogenic organisms responsible for infections, thereby rationalizing its ethnomedicinal use in the treatment of ailments. Some identified secondary metabolites could be responsible for the observed activities. Since phytochemical survey is the first step towards the discovery of useful drugs, *Pennisetum purpureum* (Schumach) can be utilized for medicinal and therapeutic purposes.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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