



Antioxidant Activities of the Aqueous Extracts of *Pedaliium murex* D. Royen EX L. Fruit and Leafy Stem

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Authors' contributions

This work was carried out in collaboration among all authors. Author GBAD carried out all the experiment, the collection of data and wrote the first draft of the manuscript. Authors GH and FA managed the quantitative phytochemical analysis and performed the statistical analysis. Author AG wrote the protocol and designed the study. Author JG managed the antioxidant assays. Author LA supplied a survey of the study and corrected the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The leafy stem and fruit of *P. murex* have been reported to be used in folk medicine to treat male reproductive system ailments. This study was undertaken to assess the antioxidant potential of the aqueous extracts of *P. murex* leafy stem and fruit.

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Methodology: Extracts were prepared by macerating the powder in water. Total phenolics amount were determined by Folin-Ciocalteu reagent, flavonoids were quantified by aluminum chloride method and total tannin content was estimated by hexacyanoferric method. The *in vitro* antioxidant activity of the extracts were assessed through 2,2'-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, reducing power assay, hydrogen peroxide (H₂O₂) inhibition assay and lipid peroxidation assay.

Results: Total phenolic compounds, flavonoids and tannins content were respectively equal to 48.91± 1.67 µg/mg Gallic Acid Equivalents (GAE); 56.01± 0.90 µg/mg Rutin Equivalents (RUE); 16.92± 1.22 µg/mg Tannic Acid Equivalents (TAE) for the leafy stem extract while they were equal to 26.26± 0.54 µg/mg GAE; 47.88± 2.39 µg/mg RUE; 7.94± 0.31 µg/mg TAE for the fruit. The leafy stem extract exhibited a more pronounced DPPH scavenging activity, reducing power, hydrogen peroxide scavenging activity and lipid peroxidation activity than the fruit extract.

Conclusion: The antioxidant activity of the leafy stem aqueous extract was significantly more potent than that of the fruit extract. Further studies will find out the extracts pharmacological efficacy and innocuity.

Keywords: Oxidative stress; Pedalium murex; phenolics content, antioxidant.

1. INTRODUCTION

Oxidative stress is the imbalance between antioxidants and prooxidants in the organism. It is due to the excess production of reactive oxygen species and/or to the decrease of antioxidant defense [1]. This phenomenon affects all the body systems and lead to various illness such as cancer, aging, atherosclerosis, Parkinson's disease and male infertility [2,3].

Infertility is defined as the failure to establish a clinical pregnancy after 12 months of regular, unprotected sexual intercourse[4]. Male infertility has various etiologies that can be grouped into pre-testicular, testicular, post-testicular and idiopathic causes. However, oxidative stress phenomenon takes place in the unfolding of male infertility especially idiopathic infertility which is characterized by the impairment of semen quality with unknown etiology. The treatment of male idiopathic male infertility relies on several off label medicine like the use of antioxidants [5,6]. The main antioxidants frequently prescribed during male infertility treatment are vitamins C and E, carnitine, carotenoids, cysteine, micronutrients such as zinc and folate [7].

Nowadays, there is a regain of interest in the use of complementary and alternative medicine in both developed and developing countries. In developed countries, the use of herbal medicine is associated with people hope to have a longer and healthier life. For developing countries, the use of medicinal plants is due to cultural reasons [8]. Herbals antioxidant sound attractive due to a misconception supporting that natural products are more safer and less toxic than their

synthetics counterparts [9]. The use of plants with antioxidant properties dates back to ancient Egypt era [10]. Some medicinal plants of African pharmacopeia with antioxidant activities have been also already described [11].

Pedalium murex is a plant of the Pedaliaceae family. It is found near the coasts in tropical regions of the world in Asia, South America and Africa [12]. Its leafy stem and fruit are frequently used in Benin folk medicine to heal several diseases such as gonorrhoea, ulcer, diabetes, and male infertility. The aqueous extracts of the leafy stem or fruit of *P. murex* are used indistinctively to treat semen quality impairment. However, to our knowledge, no study has assessed their comparative antioxidant potential. Therefore, the purpose of this work is to evaluate the antioxidant activities of the aqueous extracts of the leafy stem and fruit of *P. murex*.

2. MATERIALS AND METHODS

2.1 Plants Materials

Pedalium murex was collected in June 2019 from Ahozon and Ouidah (Benin). It was recognized and authenticated by the Benin National Herbarium where the Voucher specimen was deposited (YH 240/HNB).

2.2 Preparation of Plant Extracts

The samples were washed three times with tap water after removal of the roots. Before drying, the fruits were separated from the leafy stem. The dried fruits and leafy stems were crushed

and powdered using a sample mill (Restch SM 2000). Extracts were prepared by macerating the powder in distilled water for 24h under continuous stirring on an orbital magnetic stirrer (VWR). The mixture is then filtered three times on hydrophilic cotton. The filtrate was evaporated with a rotary evaporator (IKA-RV8) under reduced pressure then in an oven at 40°C. The yields of extraction were equal respectively to 16.67% and 9.35% for the leafy stem and fruit. The extracts were kept in separate clean glass bottles and stored at 4°C until use.

2.3 Chemicals

2.3.1 Phytochemistry

Bismuth (III) subnitrate (Merck) Potassium Iodide (VWR), Mercury (II) chloride (VWR), Chloroform (Fisher Scientific), Sulfuric acid (Fisher Scientific), Ammonia (CDH), Ferric chloride (Lobachemie), Ascorbic acid, Rutin (VWR), Gallic acid (VWR), Absolute ethanol (Merck), Folin-ciocalteu reagent (VWR), Aluminium chloride (CDH), Potassium ferricyanide (Alfa Aesar GmbH).

2.3.2 Antioxidant activities

2,2'-diphenyl-1-picrylhydrazyl (Merck), trichloroacetic acid (Central Drug House), Ascorbic acid, Rutin (VWR), Gallic acid (VWR), Hydrogen peroxide (Scharlau), Ammonium sulphate (Lobachemie), Phenanthroline (VWR), Acetic acid (Fisher Scientific), Sodium dodecyl sulfate (Merck), Thiobarbituric acid (Merck).

2.4 Determination of Total Phenolic, Flavonoids, Tannins Content

2.4.1 Determination of total phenolic content

The content of phenolics in the extracts were determined by Folin-ciocalteu method [13]. 125 µl of the extracts (2 mg/mL) were added to 625 µl of diluted Folin-ciocalteu reagent (1:10). After 5 minutes, the reaction was neutralized with 500 µl of saturated sodium carbonate (75 g/L). Subsequently, the mixture was incubated in darkness for 2h at room temperature, and then its absorbance was measured at 760 nm with a spectrophotometer. Gallic acid (45-500µg/mL) was used as standard for the calibration curve. Assays were performed in triplicate. Total phenolic content was expressed as µg Gallic acid Equivalents (GAE)/mg of dry extract.

2.4.2 Determination of flavonoids content

The flavonoid content of the extracts were quantified by aluminum chloride (AlCl₃) method [14]. 500 µl of the extract (2 g/mL) were added to equal volumes of an aqueous solution of 2 % AlCl₃. The mixture was shaken, and the absorbance was read at 420 nm after incubation in the dark at room temperature for 10 minutes. Rutin (15–500 µg/mL) was used as standard for the calibration curve. Assays were performed in triplicate. Flavonoids contents were expressed as µg Rutin Equivalent (RE)/ mg of dry extract.

2.4.3 Determination of total tannin content

Total tannin amount in the extracts was determined using the method described by Price and Butler [15]. 500 µL of the extracts were made up with distilled water to 8 mL, then 0.5 mL of 0.1M of ferric chloride (FeCl₃) and 0.5 mL of 8 mM potassium ferricyanide [K₃Fe (CN)₆] were added and incubated at room temperature for 10 minutes. The absorbance was recorded at 720 nm via spectrophotometer. Tannic acid was used as standard, and the results were expressed as µg tannic acid equivalent (TAE)/mg of extract. Assays were performed in triplicate.

2.5 Antioxidant Activity

2.5.1 2,2'-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay

Radical scavenging activity of the extracts against DPPH was determined according to the method reported by Akomolafe [16]. 1 mL of different concentrations of each extract (0-500 µg/mL) or standard (ascorbic acid) were added to an ethanolic solution of DPPH (1 mL; 0.04 mg/mL). The samples were incubated for 30 minutes in the dark and the absorbance was read at 517 nm. The percentage of inhibition of DPPH was determined according to the following formula: % Inhibition = [(Abs Control-Abs Test)/Abs Control] x 100 where Abs Control is the absorbance of the DPPH + Ethanol; Abs Test is the absorbance of DPPH + extract or standard. The assays were performed in triplicate.

2.5.2 Reducing power assay

The extracts ability to reduce Fe³⁺ was assessed according to Oyaizu method [17]. 1 mL of the extracts was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide [K₃Fe(CN)₆]. After 20

minutes of incubation at 50°C, 2.5 ml of trichloroacetic acid (10%) were added to the mixture and centrifuged for 10 minutes (3000 r/t). A 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1 %). The absorbance was read at 700 nm. Ascorbic acid was used for the calibration curve and Rutin (200 µg/mL) as standard. Ferric iron (Fe³⁺) reducing activity was determined as Ascorbic acid equivalents (mmol Ascorbic acid/g extract). The assays were performed in triplicate.

2.5.3 Hydrogen peroxide scavenging assay

Hydrogen peroxide (H₂O₂) scavenging ability of the extracts was determined according to 1,10 phenanthroline method [18]. 0.25 mL of ferrous ammonium sulfate were added to 1.5 mL of extracts or standard. Thereafter, 62.5µL of hydrogen peroxide (5mM) were added and incubated for 5 minutes in dark at room temperature. After incubation, 1.5 mL of 1,10 phenanthroline (1mM) was added to each tube and incubated for 10 minutes at room temperature. Absorbance was read at 510 nm using a spectrophotometer. Ascorbic acid was used as standard. Hydrogen peroxide scavenging percentage was calculated according to the following formula: H₂O₂ scavenging activity (%) = [(Abs sample)/Abs Blank] x 100 where Abs sample are the absorbance of solutions containing extracts or standard, ferrous ammonium sulfate and hydrogen peroxide; Abs blank is the absorbance of solution containing ferrous ammonium sulphate and 1,10-phenanthroline. The assays were done in triplicate.

2.5.4 Lipid peroxidation test

Thiobarbituric Acid-Reactive Substances (TBARS) assay described by Sadiq [19] was used to measure the lipid peroxide formed using egg-yolk homogenates for lipid source. Egg homogenate (250 µl, 10% in deionized water) and 50 µl of the extracts at different concentrations (0-1000 µg/mL) were mixed in a test tube and 200µl of distilled water was added to the tube. 25 µl of FeSO₄ (0.07 M) was added to the mixture and incubated for 30 minutes at 25°C. Thereafter, 750 µl of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% TBA (prepared in 1.1% sodium dodecyl sulfate) and 0.05 ml 20%TCA were added, vortexed and heated in a boiling water bath for 60 minutes. The tubes were cooled with iced water, then 3.0 ml of n-butanol

was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm. For the blank, 0.1 ml of distilled water was used in place of the extract.

2.6 Statistical Analysis

All experiment were performed in triplicate. Results were expressed as mean± SEM (Standard Error on the mean). Data were subject to One-way analysis of variance (ANOVA) and followed by Tukey post hoc test for multiple comparisons. Statistical significance of difference was set at p=.05. Analysis was done by GraphPad Prism 5.0.

3. RESULTS AND DISCUSSION

3.1 Total Phenolics, Flavonoids and Total Tannins Content

The quantitative determination of phenolic content in the extracts revealed that the leafy stem aqueous extract contained significant more phenolics, flavonoids and tannins than the fruit extract (Fig. 1).

3.2 Antioxidant Activities

3.2.1 DPPH radical scavenging capacity

The aqueous extracts of *P. murex* leafy stem and fruit showed concentration-dependent DPPH scavenging ability. The aqueous extract of the leafy stem exhibited a higher DPPH scavenging activity than the fruit. However, the extracts abilities to scavenge DPPH was less marked as compared to ascorbic acid (Table 1).

3.2.2 Lipid peroxidation inhibition

The extracts also demonstrated capabilities to inhibit lipid peroxidation in concentration dependent manner. However, the leafy stem extract ability to inhibit lipid peroxide formation was found more effective when compared to the fruit (Table 1).

3.2.3 Reducing power

The ferric iron reducing power of the extracts is delineate on Fig. 2. The aqueous extract of *P. murex* leafy stem was significantly more active than the fruit extract. Rutin exhibited a more potent reducing power than both extracts.

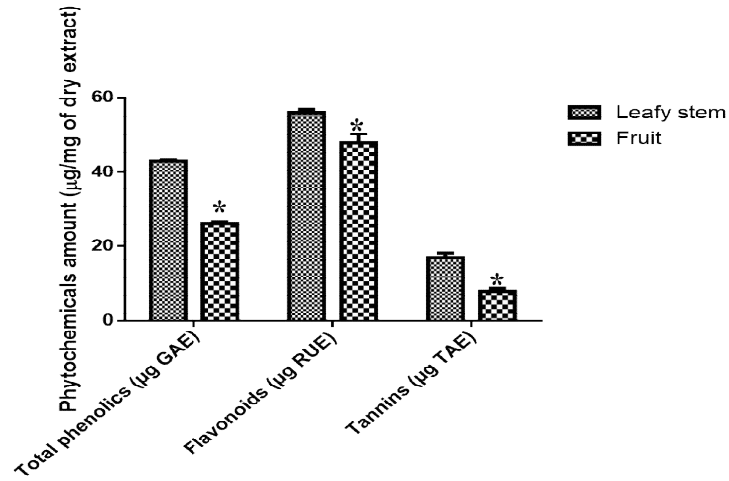


Fig. 1. Phenolics compounds amount in aqueous extracts of *P. murex* leafy stem and fruit
 * indicates that this value is significantly different from that of the leafy stem at $p = .05$

Table 1. Aqueous extracts of *P. murex* leafy stem and fruit antioxidant activities

Extracts	DPPH IC ₅₀ (µg/ml)	Lipid Peroxidation inhibition Assay IC ₅₀ (µg/ml)
Leafy stem	15.72±0.72 ^a	203.4±2.01 ^a
Fruit	32.72±1.41 ^b	717±4.5 ^b
Ascorbic acid	0.315±0.02 ^c	-
Rutin	-	285.9±6.85 ^b

Each value is expressed as mean ± standard error on the mean (n=3). Means with different letters in the same column are significantly different ($p = .05$). IC₅₀: Minimum inhibitory concentration of the extracts or standard which induce 50 % inhibition in free radical activity

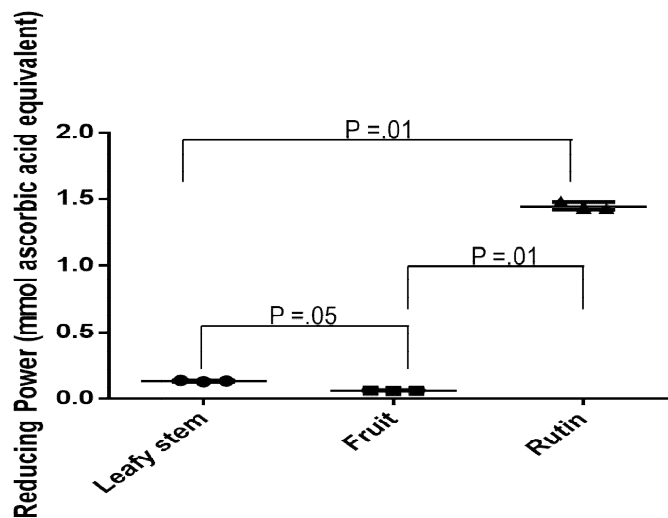


Fig. 2. Ferric iron reducing power of the extracts

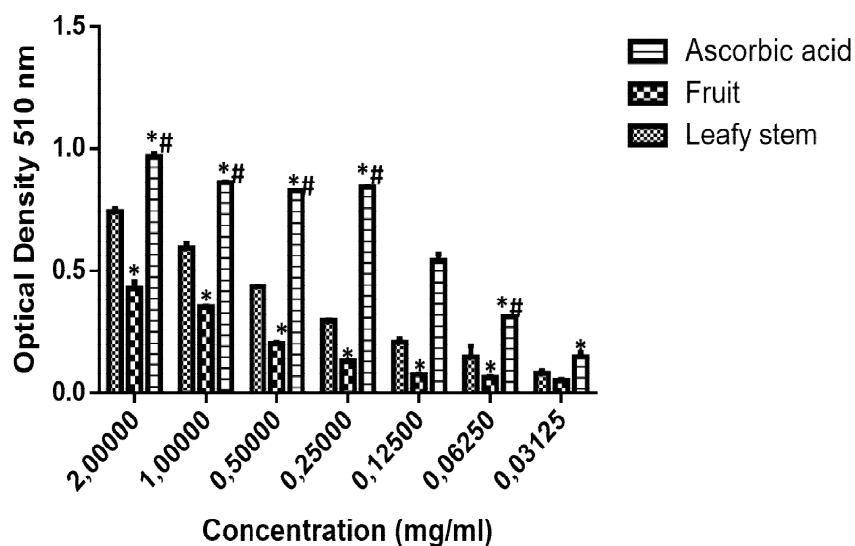


Fig. 3. Hydrogen peroxide scavenging activity of the extracts

*indicates values that are significantly different from that of ascorbic acid ($p=.05$) and #represents values that are significantly different from that of leafy stem ($p=.05$)

3.2.4 Hydrogen peroxide scavenging activity

Both aqueous extracts of leafy stem and fruit of *P. murex* displayed hydrogen peroxide scavenging capacity (Fig. 3). However, the leafy stem extract ($IC_{50}=314.3 \pm 5.50 \mu\text{g/ml}$) activity against hydrogen peroxide was more active when compared to the fruit extract ($IC_{50}=1525 \pm 35 \mu\text{g/ml}$) and ascorbic acid ($IC_{50}=407.2 \pm 7.13 \mu\text{g/ml}$).

4. DISCUSSION

Oxidative stress is the pivotal mechanism leading to the development of several metabolic and neurodegenerative diseases. The study of antioxidant activities of medicinal plants has recently gained much attention because of their potential beneficial health effects [20].

The aim of this work was to investigate the antioxidant properties of the aqueous extracts of the leafy stem and fruit of *P. murex*. The study showed that the aqueous extract of leafy stem exhibits a more potent antioxidant activities than the fruit extract. More so the total phenolic content flavonoids and total tannin amount were higher in the leafy stem extract as compared to the fruit.

Antioxidants interact with free radicals via three main mechanisms including Hydrogen Atom

Transfer (HAT), Single Electron Transfer (SET) and transition metal chelation [21]. In this study, the antioxidant activity of the aqueous extracts of the leafy stem and fruit of *P. murex* were assessed through four *in vitro* assays, namely DPPH inhibition, Ferric ion reducing power, Hydrogen peroxide inhibition and Lipid peroxidation inhibition.

DPPH assay is used to assess the free radicals scavenging ability of compounds. This assay has a good sensitivity since it can determine antioxidant power of weak antioxidant [21].

DPPH is purple in color and absorbs strongly at 517 nm. It fades to yellow after reduction by a hydrogen atom released by an antioxidant molecule. The leafy stem and fruit aqueous extracts of *P. murex* were able to scavenge DPPH in a concentration dependent manner. This data is in line with those of Thakkar [22] and Swaminathan [23] who had previously respectively revealed the DPPH scavenging activities of *P. murex* fruit and leaves. Moreover, the aqueous extract of the leafy stem was found more effective against DPPH than the fruit. This difference could be linked to their difference in phenolic compounds amount and structure [24].

The reducing power of an extract is an indicator of its potential antioxidant activity[25]. The reducing power of the extracts was assessed by

the ferric ion reduction assay. The aqueous extract of the leafy stem exhibited a more pronounced reducing power than the fruit. The extracts variable reducing power may rely on their different hydroxylation pattern and conjugation of their phenolic compounds [26]. Furthermore, the reducing power of these extracts suggests that they contain antioxidant substances able to reduce lipid peroxidation initiation rate or react and stabilize byproducts of this phenomenon [27]. These results agreed with Patel [28] which have previously showed that the polar fractions of the ethanolic extract of the fruit of *P.murex* has a higher reducing power than the apolar fractions.

Malonyldialdehyde (MDA) is a byproduct of lipid peroxidation frequently used as an indicator of this process [29]. Inhibition of MDA formation is used to assess the ability of extracts to reduce lipid peroxidation. The aqueous extracts of the leafy stem and fruit showed a concentration-dependent lipid peroxidation inhibitory activity. More so, the activity of the leafy stem extract was more marked as compared to the fruit. The effect of the extracts on lipid peroxidation could be related to their capacity to chelate ferrous ion, to reduce the formation of the iron-perferryl complex, or to scavenge hydroxyl radical [30]. The extract variable efficacy in lipid peroxidation inhibition could stem from their different content of phenolic and flavonoids [31,32].

Hydrogen peroxide is an oxidizing agent. It is involved at low level in cell signaling and differentiation [31]. Hydrogen peroxide is involved in the maturation process of spermatozoa [33]. However, cell must regulate the level of hydrogen peroxide because its excess may be detrimental to their function and survival. Both extracts have shown an ability to scavenge hydrogen peroxide in a concentration dependent manner. These effects rely on the reducing properties of their phenolic compounds able to split hydrogen peroxide into water by donating an electron [34]. These findings are in agreement with prior data of Patel [28] and Priya [35] which have showed that the fruit and leaves of *P.murex* display hydrogen peroxide scavenging activity.

The different antioxidant activities of the aqueous extracts of the leafy stem and the fruit of *P.murex* may be linked to their phenolic content. For this purpose, total phenolic compounds, total tannins, and flavonoids were determined. It was found that the leafy stem aqueous extract revealed

higher levels of phenolics, flavonoids and tannins when compared to the fruit extract.

The polyphenolic compounds of medicinal plants are well known for their antioxidant properties. They act either by scavenging free radicals or by chelating transition metals [36]. The free radical scavenging activity of these compounds is based on the reducing potential of the hydroxyl groups attached to the benzenic nucleus. The ability of polyphenols to chelate transition metals depends on the presence of hydroxyl groups in the position 3' and 4' of the B ring [37]. Flavonoids represent the most important group of phenolic compounds. They reduce the deleterious effects of oxidative stress by several mechanisms including free radical scavenging, metal chelation, inhibition of α -tocopheryl radicals, inhibition of oxidases, increased uric acid production, increased activity of low molecular weight antioxidants and activation of antioxidant enzymes [32] (13). In addition, besides flavonoids, tannins also exhibit antioxidant activities through free radical scavenging, transition metal chelation and inhibition of pro-oxidant enzymes. The antioxidant activities of tannins are more effective than several other classes of polyphenols due to their high molecular weight and the high degree of hydroxylation of their aromatic rings [38].

5. CONCLUSION

This study has shown that the aqueous extract of the leafy stem of *P. murex* possess a significantly higher antioxidant potential than the aqueous extract of the fruit. This difference in antioxidant activities of both extracts would stem from their variable amount of phenolics compounds. However, further studies are required to assess the biological activity and toxicological safety of these extracts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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