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Free Radical Scavenging Activity and Phytochemical Investigation of Ethanol Leaf Extract of *Dryopteris dilatata*

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Authors' contributions

This work was carried out in collaboration among all authors. Author AEA conceptualized the experiment. Authors AEA, JIE, DNA, AOI, AJA and IO performed the laboratory experiment and analysis all data. Authors AEA and JIE wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Bioactive constituents are the active ingredients of herbal product such as *Dryopteris dilataa* that possess therapeutic efficacies in the treatment and management of several diseases. Thus, we evaluated different concentration of the free radical scavenging activity and phytochemical constituents of ethanol and sub-extracts of *Dryopteris dilatata (Dd)* leaves such as butane, hexane and benzene. Phytochemicals analysis was determined on the ethanol extract of *Dryopteris dilatata* sub-extracts using standard principles, also free radical scavenging capacity of the ethanol extract for Ferric reducing antioxidant power (FRAP), hydroxyl radical scavenging assay (OH), superoxide radical scavenging assay (SSRA), 2,2--Diphenyl-1-Picrylhydrazyl assay (DPPH), 2, 2'-azino-bis 3-ethylbenzthiazoline-6-sulphoric acid scavenging activity (ABTS) and thiobarbituric acid (TBA) assay (TBA) was done using standard protocols. Results of the phytochemical analysis revealed flavonoids and cardiac glycosides in all extracts, terpenoid, phytosterol, alkaloid, tannins and saponin constituents were present in ethanol, butane and hexane extracts and absent in benzene extract, phenols is present in only hexane extract but absent in all other extracts, glycerols is present in only benzene extracts while phlobotannin and carbohydrate are absent in all extracts. The plant ethanol extract revealed statistically significant at *P*<0.05 capacity for (FRAP), (OH), (SSRA), (DPPH), (ABTS), (TA). The study therefore suggest that the plant extract could lead to producing therapeutic agent in the treatment and management of several disorders that are attributed to reactive species and oxidative stress.

Keywords: Bioactive compounds; phytochemicals; free radicals; antioxidant; Dryopteris dilatata.

1. INTRODUCTION

"Investigation on bioactive constituents of traditionally used plants leads to the emergence of new pharmacological agents with great therapeutic potency due to the presence of phytochemicals that are important for the treatment and management of several health conditions which include endocrine, neurodegenerative and cardiovascular disorders" [1,2]. Almost all the pharmaceutical agents available for treatment and management of several disorders were obtained from substance that are of plant origin [3]. Medicinal plants are used in developed and developing countries to treat various pathological disorders [4]. Although the important of medicinal herb is gaining global attention, not all plants constituent are used for medicinal purposes. More so, different constituent of these plants are specific for different disorder which reveals the need for proper evaluation of different phytocontituents of medicinal plants to ascertain the active ingredients that are contained in these plants for effective therapeutic recommendations [5] The ability of medicinal plants to scavenge reactive oxygen species is solely dependent on the bioactive constituents that are found in the medicinal plants. Therapeutic potency of pharmaceutical products is dependent on a particular phytochemical present in these products [6]. Examples of these phytochemicals include tannin, phytosterol, flavonoid, alkaloid, saponin, phenol, reducing sugar etc. These phytochemicals are attributed to different biological activities and are capable of combating oxidative stress by reducing excess free radicals generated in living systems. The important of these bioactive constituents cannot be ruled out in the antioxidant property of medicinal plants since they play an important role in production of synthetic drugs [7].

Free radicals that are rendered inactive by plant antioxidants include reactive oxygen and reactive nitrogen species [8]. They suppress and destroy the antioxidant mechanism and prevent them from combating oxidative stress [9]. Ailments that affect biological systems such as diabetes, cancer, cardiovascular disorders, Parkinsonism, Alzheimer's, disorders have their origin from oxidative stress which results from excessive generation of free radicals suppressing the antioxidants mechanism of biological systems [10]. One of the plants with proven medicinal properties is *Dryopteris dilatata* (Dd) [10-14].

"Dryopteris dilatata is also known as broad buckler fern, belonging to the family of dryopteridecea. The plant is distributed mainly in the tropical region of Africa particularly in Nigeria and is use traditionally for the treatment of several diseases such as diabetes and gastrointestinal disorders" [15]. Reports from several researchers revealed that *Dryopteris dilatata* possess a great deal of biological and

pharmacological activities which includes antidiabetic, hypolipidemic, hepatonepro protective activities [16]. Previous study in our laboratory [17] showed that ethyl acetate extracts of *Dryopteris dilatata* possess phytochemical constituents and antioxidant properties that are associated to its pharmacological importance [15]. The ethanolic leaf extract of the plant was reported to show significant anti-diabetic and lipid reducing activity in alloxan induced diabetic rats [15]. Although the ethyl extract of *Dryopteris dilatata* have been reported [17] there is paucity of information on free radical scavenging activity of its ethanol extract. Therefore, the present study was aimed at evaluating the comparable phytochemical constituents of different extracts of the plant and the in-vitro antioxidant capacity of ethanol extract of *Droypteris dilatata* leaf.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification and Extraction

Fresh of *Dryopteris dilatata* were obtained from its growing habitat within olomoro community in isoko south local government area of delta state Nigeria. The leaves was identified and authenticated at the Forestry research institute Ibadan with specimen number FHI 110338. The collected leaves were washed and air dried at room temperature, grinded to fine powder and was extracted using ethanol for crude extract and subsequent sub-extraction using butane hexane and benzene solvents according to methods of Ajirioghene *et al.,* (2018) [18].

2.2 Qualitative Phytochemical Analysis

The extracts of the plant were subjected to different kinds of phytochemical tests to investigate the presence of bioactive compounds namely; tannins, terpenoids, glycosides, phenols, flavonoids, steroids, saponins, alkaloids, carbohydrates, proteins, amino acids, phlobotannins, cardiac glycosides, according to their various standard methods [15,16,19,20] .

Detection of alkaloids: “Extracts were dissolved individually in dilute Hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids. Mayer’s Test: Filtrate was treated with Mayer’s reagent (Potassium Mercuric iodide). 1.5 ml of 2 percent HCl used to dissolve of extracts and later added few drops of Mayer’s reagent. Formation of a yellow cream precipitate indicates the presence of Alkaloids” [21].

Detection of glycosides: “Extracts were dissolved with dil. HCl, and then subjected to test for glycosides. Keller-kilani test: One gram each of the crude extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides” [22].

Detection of steroid: “One gram of the crude extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added likewise. A red colour produced in the lower chloroform layer indicated the presence of steroids” [23].

Detection of terpenoids: “One gram of the Crude extract was dissolved in 2 ml of chloroform and evaporated to dryness. To this, 2 ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids” [24].

Detection of saponins: Froth Test: “Extract was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins” [25].

Detection of triterpenoids: “Ten milligram (10 mg) of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added followed by addition of 2 ml of Concentrated H_2SO_4. Formation of reddish violet colour indicates the presence of triterpenoids” [26].

Detection of phenols: Ferric Chloride Test: “Extract was treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols” [27].

Detection of tannins: “Twenty ml of water was added to 5 g of each extract in a test tube and filtered. Ferric chloride solution was added to the filtrate, formation of blue-black colour indicated the presence of tannin” [28].

Detection of flavonoids: Lead acetate Test: “Extract was treated with few drops of lead acetate solution. Formation of a yellow colour precipitate indicate the presence of flavonoids” [29].

Test for Carbohydrates: Iodine Test: "One gram of Crude extract was mixed with 2 ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate" [25].

Test for Phytosterol: "One gram of the extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether.

The ether layer was evaporated and the residue was tested for the presence of phytosterol. The residue was dissolved in few drops of diluted acetic acid; 3 ml of acetic anhydride was added followed by few drops of Concentrated H_2SO_4. Appearance of bluish green colour showed the presence of phytosterol" [27].

Test for Phlobatannins: "One gram of the extract was added to 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as evidence for the presence of phlobatannin" [29].

2.3 *In-vitro* Antioxidant Activities of *Dryopteris dilatata*

2.3.1 Thiobarbituric acid (TBA) assay

The method of Göksel et al., [30] and Lins et al., [31] was used with slight modification for the determination of free radicals present in the ethanol extract of *D. dilatata* of TBA activity. Different concentrations of the final sample concentration of 0.02% w/v from the same samples prepared for FTC assay was used. Two millilitres of 20% trichloroacetic acid and 2 ml of 0.67% of thiobarbituric acid were added to various concentrations (250-3.90 mg/ml) of sample solution from the FTC method. The mixture was placed in a boiling water bath for 10 min and then centrifuged after cooling at 3000 rpm for 20 min. The absorbance activity of the supernatant was measured at 552 nm and recorded using spectrophotometer (Thermo Scientific UV1)

2.3.2 DPPH (2,2--Diphenyl-1-Picrylhydrazyl) assay

The method of Chandrika M Liyana-Pathirana [32], El Omari et al., [33] was used for the determination of scavenging activity of ethanol extracts of *D. dilatata* against DPPH free radical. Several concentrations of the plant extract was added to 1ml of 0.135 mM DPPH solution and mixed with 1.0 ml of ethanol and ethyl acetate extract The reaction mixture was mixed thoroughly and left in the dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 517 nm.

2.3.3 ABTS (2, 2'-azino-bis 3-ethylbenzthiazoline-6-sulphoric acid) scavenging activity

The method of El Omari et al., [33] and Ashafa et al., [34] was adopted for the determination of ABTS activity of ethanol extract of *D. dilatata*. The working solution was prepared by mixing two stock solutions of 7 mM ABTS solution and 2.4 mM potassium persulphate solution in equal amount and allowed to react for 12 h at room temperature in the dark. The resulting solution was diluted by mixing 1 ml of freshly prepared ABTS with various concentrations of the plant extract and measured spectrophotometric method at absorbance at 734 nm after 7 min.

2.3.4 Hydroxyl radical scavenging assay

The assay was performed according to a standard method of Salah et al., [35] and Arika et al., [36]. Quantification of the degradation product of 2-deoxyribose by condensation with TBA is the basic principle behind the assay. A reaction mixture contained, in a final volume of 1 ml, 2-deoxy-2-ribose (2.8 mM); KH_2PO_4-KOH buffer (20 mM, pH 7.4); $FeCl_3$ (100 μM); EDTA (100 μM); H_2O_2 (1.0 mM); ascorbic acid (100 μM) and various concentrations ethanol extract of *D. dilatata*. The reaction mixture was kept in incubation for 1 h at 37°C and after incubation 0.5 ml of the reaction mixture was mixed with 1 ml 2.8% trichloro acetic acid (TCA) and 1 ml 1% aqueous thiobaturic acid (TBA) was added to it, incubated at 90°C for 15 min. Thereafter incubation the solution was cooled and the absorbance was measured at 532 nm against an appropriate blank solution. All tests were performed six times. Mannitol which is a classical OH scavenger was used as a positive control.

2.3.5 Superoxide radical scavenging assay

This experiment was performed based on the reduction of nitro blue tetrszolium (NBT) according to a previously reported method of Salah et al. [35] and Arika et al., [36] with slight modification. The assay was based on the ability of the plant extract reduce nitroblue tetrazolium (NBT) into a purple-coloured formazan. The 1 ml reaction mixture contained phosphate buffer (20

mM, pH 7.4), NADH (73 µM), NBT (50 µM), PMS (15 µM) and various concentrations (250–3.9 µg/ml) of sample solution. The reaction mixture was incubated at room temperature for 5 min. Thereafter, the absorbance was taken at 562 nm against an appropriate blank solution. All tests were performed six times.

2.3.6 Measurement of ferric reducing power

The method described by El Omari et al., [33] and Oyaizu [36], was followed with slight modification to determine the Fe3+ reducing power of the ethanol extract of *D. dilatata.* Different concentrations of extract were mixed with 0.5-ml phosphate buffer (pH 6.6) and 0.5 ml 0.1% potassium hexacyanoferrate. The solution was incubated at 50 °C in a water bath for 20 min. 0.5 ml of trichloro acetic acid (TCA) (10%) was added after incubation to terminate the reaction. The upper portion of the solution (1 ml) was mixed with 1 ml distilled water and 0.1 ml FeCl3 solution (0.01%) was added. The mixture was left to stand for 10 min at room temperature and the absorbance was measured at 700 nm using spectrophotometer against an appropriate blank solution. All tests were performed six times.

2.4 Data Analysis

Data were presented as Mean ± SEM (standard Error of Mean). Results were analysed using one-way analysis of variance (ANOVA), followed by post Hoc Fisher's test (LSD) for multiple comparison and $P < 0.05$ were considered statistically significant.

3. RESULTS

Phytochemical analysis on butane, hexane, benzene and ethyl acetate fraction of *Dryopteris dilaatata* leaves.

The results of the qualitative phytochemical analysis (Table 1) for ethanol and sub-extracts of *D. dilatata* leaf revealed that alkaloids and flavonoids were present in all extracts, cardiac glycosides is present in ethanol and hexane extract, tannin and saponnin are present in ethanol, butane and hexane extract, steroids is present in ethanol and benzene extract, terpenoid is present in ethanol, hexane and butane extract and phenol is present in ethanol and hexane extract while carbohydrate and phlobotanin are absent in all extracts.

Table 1. Qualitative phytochemical analysis

Phytochemicals	Ethanol Extract	Butane Sub-Extract	Hexane Sub-Extract	Benzene Sub-Extract
Phenols	+++	-	+++	-
Steroids	+	-	-	+++
Tarpenoids	+	+++	++	-
Carbohydrates	-	-	-	-
Alkaloids	++	+	++	+
Phlobotannins	-	-	-	-
Cardiac glycosides	++	-	++	-
Flavonoids	+	+	+	+
Tannins	++	++	++	-
Saponins	++	+	++	-

Key: (+) Week, (++) present, (-) Absent

Table 2. Ferric reducing properties scavenging potential

CONC. (mg/ml)	Ethanol	Ascorbic acid
250	56.38±0.79 [a]	70.05±0.07 [c]
125	45.58±1.05 [a]	60.22±0.33 [c]
63.5	26.93±0.49 [a]	54.68±0.58 [c]
31.5	20.69±0.30 [a]	45.16±0.06 [c]
15.25	16.56±0.29 [a]	28.38±0.55 [c]
7.625	3.18±0.29 [ac]	4.55±0.17 [c]
3.825	0.96±0.06 [ac]	1.62±0.27 [c]

Values are expressed as mean ± SEM. ANOVA followed by Post Hoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at P < 0.05

It was observed that concentrations of ethanol extract of *Dd* showed statistically significant (µ/ml) scavenging ferric reducing power compared to the control ascorbic acid.

Table 3. Hydroxyl (OH) radical scavenging potential

Conc. (mg/ml)	Ethanol	Manitol
250	80.89±0.10 [a]	9.20±0.24 [c]
125	66.29±0.08 [a*]	86.92±0.38 [c]
62.5	53.79±0.49 [ac]	68.80±.42 [c]
31.25	46.23±0.42 [ac]	54.26±0.89 [c]
15.625	23.66±0.10 [ad]	38.38±0.29 [c]
7.825	9.34±.31 [ad]	26.61±0.18 [c]
3.906	80.89±0.10 [ae]	14.97±0.50 [c]

Values are expressed as mean ± SEM. ANOVA followed by Post Hoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at P<0.05

At higher concentrations ethanol extract of *Dd* showed statistically significant hydroxyl radical scavenging activity compared to the standard antioxidant manitol.

Table 4. ABTS radical scavenging potential

Concentration	Ethanol	Sodium Ascorbate	Trolox	Galic acid
250	63.38±0.18 [a]	65.32±0.33 [a]	96.13±0.79[b]	67.49±.72 [a]
125	54.30±0.31 [a]	57.10±0.51 [a]	78.18±0.05[b]	64.85±0.32 [a]
62.5	44.06±0.68 [ac]	49.18±0.55 [ac]	48.62±0.30 [ac]	59.85±0.57[b]
31.25	33.09±0.06 [ac]	20.04±0.55 [ac]	30.38±0.38 [ac]	56.23±0.27[b]
15.655	27.04±0.52 [ad]	7.70±0.17 [ae]	14.36±0.22 [ae]	44.42±0.31[b]
7.825	20.09±0.67 [ae]	4.62±0.26[c]	9.23±0.39[c]	14.11±0.37[c]
3.908	-9.24±0.31[o]	1.84±0.10[*]	4.47±0.21[*]	6.44±0.40[*]

Values are expressed as mean ± SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at P<0.05

The maximum scavenging activity for ethanol extract for ABTS was recorded for higher concentrations compared to the reference antioxidants, and this activity decreased as the concentration reduces.

Table 5. DPPH radical scavenging activity

Concentration	Ethanol	Ascorbic acid	Tocophenol
250	59.46±0.53 [a]	56.56±0.39 [a]	67.37±0.25 [b]
125	49.09±0.14 [a]	50.09±0.23 [a]	58.01±0.42 [b]
62.5	41.20±0.43*	47.14±0.61 [*]	47.78±0.41 [*]
31.25	33.52±0.22 [*]	44.22±0.67 [*]	39.37±0.35 [*]
15.625	25.26±0.89 [*]	35.75±0.59 [*]	27.95±0.29 [*]
7.825	19.13±0.01[*]	19.12±0.79 [*]	11.64±0.63 [*]
3.906	7.76±0.37[0]	3.20±0.26 [d]	4.28±0.22 [d]

Values are expressed as mean ± SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at P<0.05

A maximum scavenging activity for DPPH radicals was recorded at concentrations for ethanol compared to the standard antioxidant ascorbic acid and tocopherol respectively. This activity decreases as the concentration decreased.

Table 6. Superoxide radical scavenging (SRS)

Conc. (mg/ml)	Ethanol	Ascorbic acid
250	69.69±1.12 [a]	59.77±0.34 [b]
125	54.65±0.60 [a]	45.15±0.56 [b]
62.5	38.99±0.16 [a]	38.46±0.82 [b]
31.25	28.97±0.98 [a]	25.81±0.51 [b]
15.825	18.27±0.62 [a]	14.29±0.09 [b]
7.825	9.76±0.61 [a]	9.36±0.14 [b]
3.906	3.39±0.29 [ad]	6.59±0.12 [bd]

Values are expressed as mean ± SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at P<0.05

Ethanol extract of *D. dilatata* was observed to inhibit superoxide generation in concentration dependent manner with the maximum scavenging activity recorded in 250 µg/ml for ethanol extract compared to the reference antioxidant which declined as the concentration reduces.

Table 7. Thiobabituric Acid (TBA) scavenging activity

Concentration	Ethanol	Ascorbic acid
250	76.32±0.79 [a]	85.19±0.79 [c]
125	61.62±0.23 [a]	70.48±0.23 [c]
62.5	41.53±0.35 [a]	50.39±0.35 [c]
31.25	28.37±0.13 [a]	37.24±0.13 [c]
15.625	15.60±0.67 [ab]	17.47±0.67 [c]
7.825	6.50±0.04 [abc]	8.36±0.04 [c]
3.906	1.07±0.44 [a*]	2.17±0.46 [c]

Values are expressed as mean ± SEM. ANOVA followed by PostHoc (LSD) multiple range tests. Values not sharing a common superscript differ significantly at P<0.05

The results of Thiobabituric acid scavenging activity for ethanol extract of *D. dilatata* in Table 7 is shown above. Ethanol extract showed a statistically significant scavenging activity for TBA compared to the standard ascorbic acid.

4. DISCUSSION

Medicinal plants containing important phytochemicals that ameliorate several pathological conditions are now searched for in developing countries, as almost all the different parts of medicinal plants have in their distinctive potency in the reversal of the deleterious activity seen in various disease states which owes their therapeutic activity to the presence of the active ingredients present in the herbal remedy used [37,38]. The presence of these phytochemicals in the plant extract correlates with reports from other medicinal plant phyto constituents that are of great medicinal value [37]. Phyto constituents that have been observed to be present in *D. dilatata* leaf extract in the present study can be associated to the plants in-vitro-antioxidants capacity observed in this study.

Health benefits of natural product containing antioxidants scavenging activity has become of global interest since a variety of destructive capability of infectious agents arise from generating oxidants leading to oxidative stress causing damage to cells, organs and the entire human systems [39,40]. "The results of DPPH showed that the ethanol extract of *D. dilatata* possess a strong antioxidant potential at higher concentrations compared to the standard but this decreases as the concentration decreases. This reveals the ability of the plant to scavenge in-vitro antioxidants. For superoxide radical scavenging activity it was observed ethanol extract of *D. dilatata* inhibit superoxide generation in a concentration depend manner, showing that at high concentration maximum inhibitory activity was observed compared to the reference antioxidant while it declined as the concentration decreases" [22,37,39].

The amount of lipid peroxidation (MDA) in living systems determines the level of oxidants that can cause oxidative damage in several pathological conditions, the capacity of plant antioxidant to scavenge Thiobarbituric acid shows the medicinal activity to the plant to ward off free radicals that can cause such damage which is seen in the antioxidant activity of ethanol extract of *Drypoteis dilatata* to donate electrons and prevent the harmful activities of reactive species thereby reversing its harmful effect showing its therapeutic capacity in various disorders which is in agreement with results from several studies on medicinal plant extracts antioxidant capacity [39-42].

This ability of ethanol extract of *Dryopteris dilatata* to reduce Fe_{3+} (ferricyanide complex) to Fe_{2+} (ferrous form) indicates that it donates electron which signifies it reducing power, this may be attributed to higher amount of phenolic content in the plant extract due to higher content of phytochemicals in the extract. This shows the antioxidant activity of plant extract as compared to other medicinal plants [31]. In the present study ethanol extract of *Dryopteris dilatata* compared to the standard compound reveals its electron donating capacity showing its antioxidant potential as a medicinal plant against free radicals [24,32].

ABTS radical scavenging activity is done to evaluate the antioxidant capacity of a plant extract in hydrogen donating activity [24]. The antioxidant capacity of ethanol extract of *Dryopteris dilatata* to scavenge ABTS in donating hydrogen is important to prevent oxidation process by converting reactive species into a more stable form as seen in previous research work on antioxidants activity of medicinal plants [39]. The present study correlates with data recorded from earlier publications on antioxidant capacity of medicinal plants that associated with the phytochemical content of the plant that enables it potency to scavenge free radical species [29,31].

Hydroxyl radical is the most potent reactive species in free radical pathology of biological systems which can cause breakage of DNA strand that is capable of damaging exclusively all cellular components leading to cell death. At higher concentration ethanol extract has higher scavenging activity compared to standard which declined at lower concentrations but was observed to be higher at the lowest concentration. "The highly reactivity activity of hydroxyl radical that is being continuously formed in a process of reduction of oxygen to water which causes lipid peroxidation is evident in its deleterious effect in cells and organs of the body" [33]. "Ethanol extract of *Dryopteris dilatata* inhibited the generation of reactive species of hydroxyl radical at different inhibition values in different concentrations as reported in other medicinal plant studies" [27,41]. The reaction consequence results in a lot of medical conditions that can be prevented by the antioxidants properties of medicinal plants [35, 41] which inhibitory activity is seen in the ethanol and ethyl acetate extract of *Dryopteris dilatata.*

An unstable free radical molecule DPPH that has a deep violet colour in solution turns into pale yellow by extracts scavenging activity for the ethanol extract being able to provide hydrogen atom donating ability that can reduce DPPH (2,2-diphenyl-2-picylhydrazyl) to DPPH-H (1,1-diphenyl-2-picylhydrazine). The natural antioxidant content of the ethanol extract of the plant extract as the inhibition of DPPH radical by the plant extract to become stable which depends on the ability of the extract to donate electrons, this reveals the antioxidant capacity of the plant extract as an electron donor which can covert free radicals into a more stable compound and reversing the deleterious effect of oxidation. This result is in line with reports from De Pooter and et Schamp, [42] who investigated the ability of the reference antioxidants ascorbic acid and tocopherol to trap DPPH due to its capacity to donate electron and reports from other plant studies that reveals the antioxidants capacity of medicinal plants to provide stability and ward off free radicals [24,39,43] implying that oxidative damage pathologies could be treated with extracts from *Dryoptreris dilatata*.

Dryoptreris dilatata due to its capacity to donate electron and reports from other plant studies that reveals the antioxidants capacity of medicinal plants to provide stability and ward off free radicals [24,39,43] implying that oxidative damage pathologies could be treated with extracts from *Dryoptreris dilatata*.

"Superoxide anion (O_2) a reactive species that is found in the human system abundantly, was observed to be inhibited by the extracts of *D. dilatata.* Ethanol extract of *D. dilatata* inhibited superoxide generation in a concentration dependent manner. In the present study the ethanol extract derived from *Dryopteris dilatata* leaf showed a significant superoxide scavenging

radical activity. Superoxide radical is one of the strongest reactive oxygen species among the free radicals. It is generated in living systems through incomplete metabolism of oxygen damage results in damage of cells components and organs" [44]. "This harmful effect to cellular components could be prevented by removing superoxide radicals" [34,44]. The result from the present study indicates that the scavenging activity of superoxide of the extract compared to the reference antioxidant increased as the concentration increased which reveals the potency of the extract to scavenge for superoxide radical and reverse its deleterious effect to cells and organs of living systems. This indicates that the plant could be efficacious [44], and could be useful in ailments such as chronic pain, inflammation and Crohn's disease, cancer (murine and cell line research models) and anecdotal claims (Alzheimer's disease and multiple sclerosis) [45].

5. CONCLUSION

The results obtained in the present study has led to the conclusion that the ethanol extract of *D. dilatata* leaf as revealed that it possess valuable amount of phytochemicals constituents such as alkaloids, flavonoids, cardiac glycosides, tanninm, saponnin, steroids, terpenoids and phenols that have been reported to be attributed to have oxidative stress ameliorative activity which was revealed in ability of the ethanol extract capacity to scavenge free radicals in-vitro compared to the reference antioxidants tocopherol, manitol and arscoibc acid. All these capacities of the plant phytochemicals and antioxidant activity enlist the plant as one with great medicinal benefit and a natural source in the treatment to several pathologies.

CONSENT

It is not applicable.

ETHICAL APPROVAL

PAMO University of Medical Sciences Animal Research Ethics Committee, which agreed with the "Guide to the Care and Use of Laboratory Animals in Research and Teaching" as prescribed in NIH publications volume 25 No.28 revised in 1996, approved the use of animals for this study with approval number PUMS-AREC/056.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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