Trends in
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In vitro anti-diabetic and antioxidant activities of ethanol leaf extract of the *Nephrolepis exaltata* (l.) Schott. (Nephrolepidaceae).

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Abstract: Diabetes is one of the non-communicable diseases growing rapidly worldwide with increasing prevalence in children adolescence and young adults. The study of alpha–amylase and alpha-glucosidase inhibitory activities of agents provides an insight of their possible antidiabetic properties. The enzymes α–amylase and α-glucosidase are responsible for postprandial glucose levels. The aim of this study was to investigate the antioxidant and inhibitory potentials of the ethanol leaf extract of *Nephrolepis exaltata* (L.) Schott. (Nephrolepidaceae) against α–amylase and α-glucosidase *in vitro*. DPPH (1,1 diphenyl-2-picryl hydrazyl) free radical scavenging activity and lipid peroxidation assay were used to assess the antioxidant assay of *N. exaltata* using ascorbic acid as standard. Alpha-amylase and alpha-glucosidase inhibitory activities were evaluated. Preliminary phytochemical screening showed the presence of phenols, tannins, flavonoids, alkaloids, resins, saponins and carbohydrate. The inhibitory concentration (IC_{50}) for DPPH assay was 5.83 µg/ml and 0.04 µg/ml for anti-lipid peroxidation assay were obtained for the radical scavenging activities, while IC_{50} of 0.05 µg/ml and 0.03 µg/ml were obtained for the α-amylase and α-glucosidase inhibitory assay respectively. The ethanol leaf extract of *Nephrolepis exaltata* possesses antioxidant activities useful and inhibited α-amylase and α–glucosidase which are responsible for postprandial glucose levels.

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INTRODUCTION

Antioxidants are needed to prevent the formation and oppose the actions of reactive oxygen and nitrogen species, which are generated in vivo and cause damage to DNA, lipids, proteins, and other biomolecules. Endogenous antioxidant defences (superoxide dismutase, H_2O_2-removing enzymes, metal binding proteins) are inadequate to prevent damage completely, so diet derived antioxidants are important in maintaining health (Halliwell, 1996). Endogenous antioxidants are produced to combat various free radicals (Sundaram *et al*., 2021). Major sources of this class of antioxidants are vegetables, fruits and grains. Other very effective sources are berries, green tea and dark chocolate (Kumar *et al*., 2019). The human body employs antioxidants to counter free radicals (Bhattacharya, 2015). Antioxidants bind with free radical by giving up their own electrons resulting in the termination of oxidative chain reactions and preventing free radicals from attacking cells. Antioxidant attains free radical state after donating its electrons. It can accommodate the change in electrons without becoming reactive and that is why they are not harmful (Atta *et al*., 2017). Oxidative stress has been implicated in various pathological conditions involving cardiovascular disease, cancer, neurological disorders, diabetes, ischemia/reperfusion, other diseases and ageing (Valko *et al*., 2007). These diseases may be divided into two groups: i) diseases characterised by pro-oxidants shifting the thiol/disulphide redox state and impairing glucose tolerance, the so-called "mitochondrial oxidative stress" conditions and ii) disease characterised by "inflammatory oxidative conditions" and enhanced activity of either NAD(P)H oxidase (leading to atherosclerosis and chronic inflammation) or xanthine oxidase-induced formation of ROS (Luc *et al*., 2019). The process of ageing is to a large extent due to the damaging consequence of free radical action (Valko *et al*., 2007). Increased oxidative stress has been proposed as one of the major factors causing hyperglycemia which in turn trigger various diabetic conditions (Singh *et al*., 2022).

Diabetes mellitus is a heterogeneous group of disorders characterized by hyperglycemia due to lack of insulin, defective insulin action, or both (Karalliedde, and Gnudi, 2016). The chronic hyperglycemia of diabetes mellitus is associated with end organ damage, dysfunction, and failure, including the retina, kidney, nervous system, heart, and blood vessels (Alam *et al*., 2014). These complications arise due to the distortion in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids and proteins emanating from defective insulin secretion, insulin action, or both (Sarkar *et al*., 2019). It is projected that by 2030, the number of people with impaired glucose tolerance would have increased to 472 million, or 8.4% of the adult population from the about 285 million people in the age group 20-79 in 2010 about 70% of whom live in developing nations. (Singh *et al*., 2019).

The debilitating effects of diabetes mellitus include various organ failures, progressive metabolic complications such as retinopathy, nephropathy, and/or neuropathy (Preguica *et al*., 2020).

Nephrolepis exaltataia is a fern commonly known as the sword fern or Boston fern. It is a species of fern in the family Nephrolepidaceae, native to tropical regions throughout the world. An evergreen perennial herbaceous plant (Dehgan, 2023), it can reach as high as 40–90 centimetres (16–35 in), and in extreme cases up to 1.5 meters (4 Ft. 11 in). It is also known as the Boston sword fern, wild Boston fern, Boston Fern, Boston Blue Bell Fern, tuber ladder fern, or fishbone fern, locally called 'Imu' in Yoruba Language (Byalt, and Korshunov, 2021).

The fern has been studied for its soil phytoremediation properties (Sultana *et al*., 2015). The effects of its volatile oil (El-Tantawy *et al*., 2016) and its possible hormonal and cytotoxic effects on human cancer cells (Raimi *et al*., 2020) has also been documented. The species is in ethnomedicine in the island of Fiji to treat women's menstrual disorders (Popovici *et al*., 2018). The quantitative phytochemical constituents of the extract of Nephrolepis *exaltata* (L.) Schott have been reported with the identification of saponins, flavonoids, tannins and reducing sugars from its extract (Oloyede *et al*., 2014).

Materials and Methods

Reagents and Instruments

Distilled water, Ferric chloride (Sigma Aldrich Germany), 10% lead acetate, Dragendorff reagent (Merck), Wagner's reagent (Merck), Mayer's reagent (CDH Fine Chemical), Hager's reagent, Barfoed reagents, Fehling's A and B reagent (Sigma Aldrich Germany), 10% naphthol solution, concentrated hydrochloric acid, hydrochloric acid (1%), concentrated and dil. sulphuric acid (BDH Binder), Acetic anhydride, 70%, 90% and absolute ethanol (EMD Milliform Corporation, Germany), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Chloroform (Merck), Glacial acetic acid (GFS Chemicals, Inc., Columbus), 3.5% ferric chloride, standard α-Amylase, α-Glucosidase, Phosphate buffer, 1% Soluble Starch, Maltose, A glucose estimation kit (GLUC-PAP) from RANDOX, Dinitro salicylic acid, Liver homogenate, Acarbose, Ascorbic acid, Trichloroacetic acid, Thiobarbituric acid (Sigma Aldrich Germany), Ferrous

sulphate (GHCL, India), Sodium bicarbonate. The assay was run using Biomate 3 Ultraviolet spectrophotometer (Rochester, New York, USA).

Plant Identification and Collection

The leaves of *Nephrolepis exaltata* (2 Kg) were collected from Tigbo-Ilu, Ado-Odo/Ota local government area of Ogun state (6 41'00 N, 3 41'00E) in August, 2020. The sample were identified as *Nephrolepis exaltata* by Mr.G.I. Nodza of the Department of Botany, University of Lagos and voucher specimen number LUH 8402 was assigned by the Lagos University Herbarium. The plant materials were air dried for 72 h and then oven dried at 40ºC to remove moisture totally. Dried plants were pulverized and stored for further use.

Extraction of plant material

About 1.4 grams of the dried powdered leaves of *Nephrolepis exaltata* was extracted with 2 litres of hydrethanol for 72 h. The extracts were filtered and concentrated using a rotary evaporator (Buchi, Switzerland) at 40 °C. The dried extracts were stored at 4 °C until ready for use. Percentage yield was calculated using the formula;

$$Yeild\ (\%) = weight\frac{extract}{dried\ material}(g) * 100\% \quad [1]$$

Phytochemical Screening

The ethanol leaf extract of *N. exaltata* was screened for the presence of sugars, alkaloids, glycosides, saponins, steroids, flavonoids, anthraquinones, cardiac glycosides and tannins by method described in Trease and Evans (2009).

Antioxidant Assay

1,1 Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay.

The antioxidant activity of the extracts was evaluated based on the free radical activity of the stable 2, 2 - diphenyl-1-picrylhydrazyl (DPPH), in comparison with ascorbic acid standard, by a modified method employed by Blois (1958). Three millilitres (3ml) of methanol mixed with DPPH solution (0.1mM, 1ml) was used as control. Similar process was carried out with ascorbic acid standard carried out with each of the two extract samples.
The formula below was used to calculate the percentage (%) Inhibition:

$$Percentage\ inhibition = \frac{Aa1-Aa2}{Aa1} X\ 100\% \quad [2]$$

Where Aa1 is the absorbance of the control and
Aa2 is the absorbance of the extract

***In vitro* anti-Lipid Peroxidation Assay**

Excised liver was homogenized with 5 volumes of cold 0.15 M KCl and the microsomal fraction was separated by centrifugation at 900 g for 15 min, in 0.05 M Tris-HCl/10% 0.15 M KCl, pH 7.4. The fraction was washed thrice in buffer by centrifugation, with subsequent sedimentation at 125000 g for 10 min. The pellet obtained was stored at 80°C until use (Laferriere *et al*., 2019). The liver microsomal fraction (0.5 mg protein/mL) was mixed with a test dose of 25, 50, 100, 200 and 500 µg/mlg/mL of each extract or fraction and incubated at 37°C for 30 min. Immediately after incubation, TBA reagent containing 0.8% TBA in 0.1 N NaOH and 7% perchloric acid, pH 7.0 was added. The solution was heated at 80°C for 10 min and to the cooled mixture, pyridine: n-butanol (3:1) and 1 N sodium hydroxide were added and the absorbance was measured at 548 nm. The % inhibition of lipid peroxidation was calculated by using Equation [2].

α-amylase and α-glucosidase Inhibition assay

Alpha (α) amylase and α-glucosidase inhibitory assay of the extracts were carried out in conformity to the standard method of Ademiluyi and Oboh, (2013) with slight modification. Serial dilutions of the extracts were made, (25, 50, 100, 200, and 500 µg/ml), 1% soluble starch was used as substrate for α-amylases and 1g maltose for α-glucosidase (both in phosphate buffer pH = 6.8, 20 mM), absorbance was measured at 540 nm using a multiplate reader (Multiska thermo scientific, version 1.00.40). Acarbose at various concentrations (25-500 µg/ml) were used as a standard. Negative control samples were also included and measured alongside test samples. All procedures were performed in triplicates. Results were expressed as percentage inhibition and 50% inhibition concentration (IC_{50}) of plant extracts against lipid peroxidation, α-amylase and α-glucosidase were calculated.

RESULTS

Phytochemical Screening

Result of the phytochemical screening of the extract revealed the presence of bioactive substances (Table1)

Antioxidant Assay

DPPH assay

The scavenging abilities of extract for DPPH (63.31%) was comparable to that of ascorbic acid (81.34%) at 2 %w/v (Figure 2).

Lipid Peroxidation Assay

The percentage peroxidase inhibitions of the extract at 80 µg/ml were 40.58% while that of ascorbic acid was 72.82% (Figure 3).

ANTIDIABETIC ASSAY

α- Amylase and α Glucosidase assay

The results revealed that the leaf extracts inhibited α-amylase and α-glucosidase in a dose-dependent manner. The extract inhibited α-amylase (69.77% ± 0.27) and α-glucosidase (48.77% ± 0.89) (Table 2).

Table 1: Phytochemical constituents of the extract

Phytochemical test	Response
Test for Saponins	Positive
Test for Alkaloids	Positive
Test for Tannins	Positive
Fehling's test	Positive
Keller Killiani test	Positive
Anthraquinone test	Positive
Kedde's test	Positive
Molisch test	Positive
Shinoda's Test	Positive

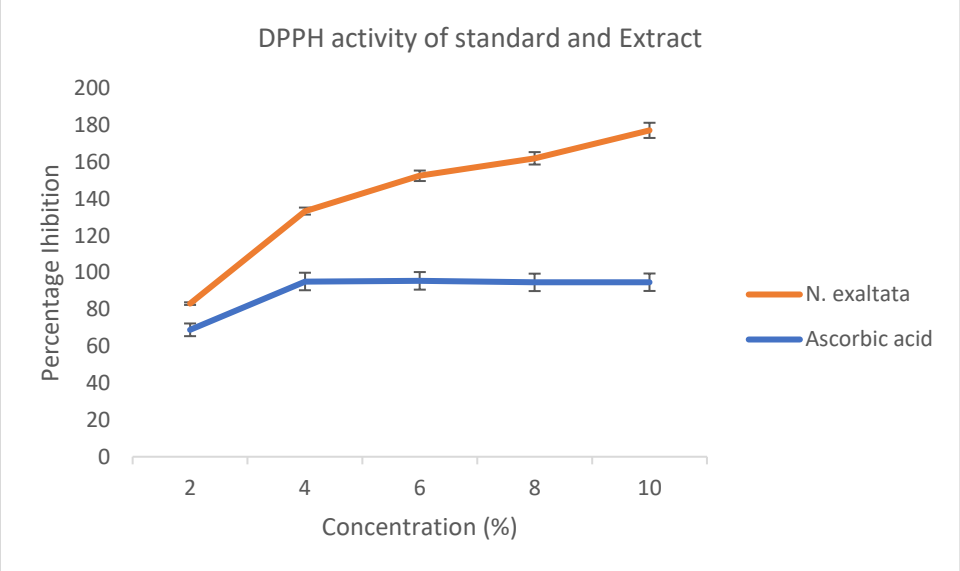

Figure 2: Percentage inhibition of extract (IC_{50} =5.833) and standard (IC_{50} =3.933) using the DPPH assay.

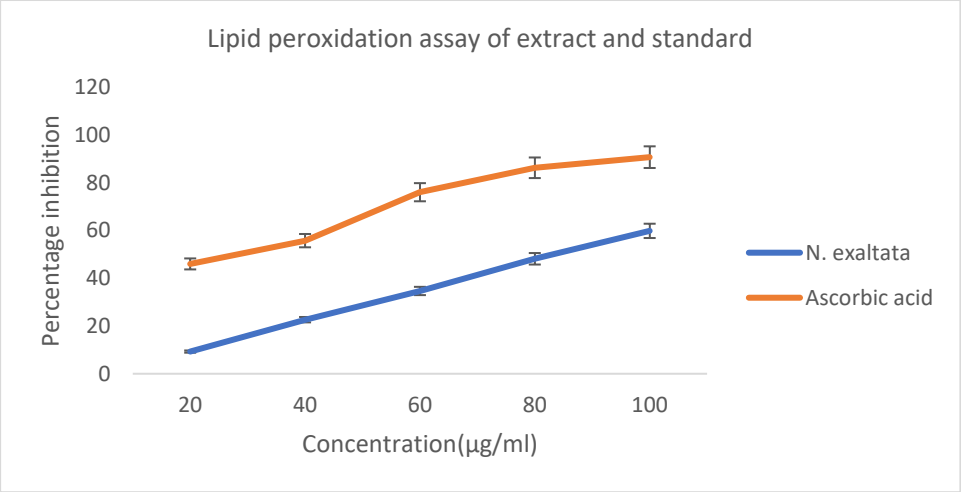

Figure 3: Peroxidase inhibition by the extract

Table 1: Phytochemical analysis of cashew nut shell extracts

Phytochemicals	**Methanol**	**Ethanol**
Terpenoids	+++	+++
Tannins	+	+
Flavonoids	+++	+++
Phenols	++	++

Key: - = absent; + = low in abundance; ++ = moderate in abundance; +++ = high in abundance

Table 2: Antidiabetic Assay of extract and standard.

	Percentage Inhibition (%) of α-amylase				
Concentration (µg/ml)	25	50	100	200	500
N. exaltata	12.96±0.98	38.54±1.69	50.28±0.56	56.48±0.81	69.77±0.27
Acarbose	61.40±0.29	77.09±0.23	80.67±0.18	81.56±0.24	82.91±0.17
	Percentage Inhibition (%) α-glucosidase				
Concentration (µg/ml)	25	50	100	200	500
N. exaltata	9.30±1.63	17.89±1.14	23.16±0.43	33.33±1.08	48.77±0.89
Acarbose	41.37±0.11	46.48±0.05	51.59±0.12	58.99±0.12	67.75±0.18

DISCUSSION

The preliminary phytochemical screening of *Nephrolepis exaltata* leaf extracts showed presence of alkaloids, tannins, flavonoids, saponins, phenolic substances, resins, carbohydrates, cardiac glycosides and anthraquinones. The free radical scavenging activity of *Nephrolepis exaltata* was tested by its ability to change colour of the stable1,1 Diphenyl-2-picryl hydrazyl (DPPH) radical. The percentage inhibition of scavenging abilities of *N. exaltata* for DPPH showed 82.40%±2.30% DPPH inhibition at 10%w/v which was the highest concentration compared to the standard (94.69%±0.20) at the same concentration. This implies that the ethanol leaf extract of *Nephrolepis exaltata* exhibits free radical scavenging activity.
IC_{50} value is the concentration that causes 50% inhibition of DPPH free radical. An increase in IC_{50} values denote a decrease in antioxidant activity, The antioxidant activity might be attributed to it poly-phenolic content and other phytochemical constituents. The extract exhibited a moderate lipid peroxide inhibition suggesting free radical scavenging activity. There was a close relationship between the antioxidant activity of the extract and ascorbic acid. The findings suggest that *Nephrolepis exaltata* could be a potential source of natural antioxidant that could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative disease as well as inhibiting lipid peroxidation. Antidiabetic activities were evaluated using α-amylase and α-glucosidase assay method. Alpha (α)–amylase and α-glucosidase is responsible for postprandial glucose levels (Ullah *et al*., 2020). Inhibition of α-amylase and α-glucosidase activities could address the major drawback and adverse effects of currently used α-glucosidase and α-amylase inhibitor drugs (Sultana *et al*., 2020). Such adverse effects might be caused by the excessive pancreatic α-amylase inhibition resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon.
This study concludes that *Nephrolepis exaltata* contains bioactive phytoconstituents that could reduce postprandial blood glucose level. Its antioxidant activity may be related to the presence of phenolic and flavonoid compounds that may scavenge free radicals that are implicated in many diseases.

Conflict of Interest
None

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