ASIAN J NAT PROD BIOCHEM
Volume 22, Number 2, December 2024
Pages: 89-97

E-ISSN: 2580-2550
DOI: 10.13057/biofar/f220205

Investigating the effect of ethanolic extract of *Dioclea reflexa* seeds on antioxidant defense, lipid profile, liver and kidney peroxidation in adult male Wistar rats

BUKUNOLA OLUYEMISI ADEGBESAN[1,♥], ESTHER NKECHI EZIMA[1], ADEFEMI OLUWASEGUN ADEFUYE[1], IFABUNMI ODUYEMI OSONUGA[2]

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University. Sagamu Campus, Ogun State, Nigeria. Tel.: +234-805-612-8331, ♥email: adegbesan.bukunola@oouagoiwoye.edu.ng

[2]Department of Physiology, Faculty of Basic Medical Sciences, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University. Sagamu Campus, Ogun State, Nigeria

Manuscript received: 1 May 2024. Revision accepted: 1 September 2024.

Abstract. *Adegbesan BO, Ezima EN, Adefuye AO, Osonuga IO. 2024. Investigating the effect of ethanolic extract of* Dioclea reflexa *seeds on antioxidant defense, lipid profile, liver and kidney peroxidation in adult male Wistar rats. Asian J Nat Prod Biochem 22: 89-97.* Several synthetic agents have been developed to combat oxidative stress associated with degenerative diseases, but factors including high cost, side effects, and lack of availability pose major setbacks in achieving the desired goal. Thus, it is therefore of utmost importance to explore the use of natural products that are less expensive and abundant in many plant sources to circumvent the alarming rates of these diseases. This study assessed the phytochemical content, the antioxidant properties of the ethanolic extract of *Dioclea reflexa* (Hook.f.) C.Wright seeds, and the effects of the extract on the lipid profile, liver, and kidney peroxidative systems of adult male Wistar rats. Therefore, 32 matured male Wistar rats (160-180) g were divided randomly into four treatment groups containing eight (8) rats each. Animals in the normal control group were treated with diluted ethanol, while those in the other three groups received an ethanolic seed extract of *D. reflexa*, 5 mg/kg, 10 mg/kg, and 15 mg/kg, respectively. After two weeks of experimental study, the animals were allowed to fast overnight before sacrifice. After that, their whole blood, kidney, and liver tissues were taken for further analysis. Assays for lipid peroxidation (liver and kidney), antioxidants, and serum lipid profile were evaluated. Our results revealed that *D. reflexa* seeds ethanolic extract contains considerable amounts of phytochemicals; significantly possesses antioxidant activities, repressed LDL and total cholesterol levels with a concomitant increase in HDL levels in Wistar rats; significantly reduced liver and kidney lipid peroxidative damage, especially at 10 and 15 mg/kg doses relative to the control rats. This study suggests that the seeds of *D. reflexa* possess antioxidative, HDL level raising, and non-HDL cholesterol lowering activities and thus may be useful in the management of lipid-associated disorders as well as hepatic and renal malfunctions.

Keywords: Antioxidants, cholesterol, *Dioclea*, lipid peroxidation, lipoprotein

INTRODUCTION

In humans, medicinal plants' therapeutic and beneficial pharmacological effects are very important and cannot be overlooked. Since the medieval age, people have utilized medicinal plants to manage a variety of illnesses and as components for the manufacture of beneficial medications. Natural products are sometimes more useful and efficient than synthetic analogs because they present fewer adverse effects, are economically affordable, and have efficacy in multidrug-resistant cases (Ye et al. 2015). Medicinal plants are now being recognized worldwide as useful tools for researchers in drug discovery, invention, and development (Chopra and Dhingra 2021; Noor et al. 2022).

According to Liguori et al. (2018), oxidative stress is well associated with the pathophysiology of numerous chronic illnesses, including atherosclerosis, cancer, Parkinson's disease, immune system dysfunction, diabetes mellitus, and aging. Concisely, oxidative stress results from an unstable or imbalanced situation between the rate at which free radicals are produced and the rate at which cells eliminate them. This imbalance can lead to lipid peroxidation, cell membranes and lipoproteins damage, and the ultimate development of mutagenic and cytotoxic compounds, such as conjugated diene and malondialdehyde (MDA).

Reduction in the risk of several diseases resulting from antioxidant activities has been attributed to phytochemicals such as flavonoids (present in fruits and vegetables), carotenoids (from carrots), alkyl sulfide (found in onions and garlic), lignans, coumarins, terpenoids, polyphenolics, plant sterols, saponins, phthalides and curcumins (Jimenez-Garcia et al. 2018). Numerous researchers have examined the potential health benefits of medicinal plants, including their hypolipidemic, hypoglycemic, antitumor, and immune-stimulating qualities, which may help to lower the onset of cardiovascular issues and cancer (Ye et al. 2015; Anwar et al. 2016; Shaito et al. 2020). However, despite the use of synthetic drugs, the prevalence of degenerative diseases, including cancer, diabetes, and hypertension, is increasing in both developed and developing nations. Therefore, investigating the usage of natural products is crucial to avoid the problematic rates at which these diseases are occurring.

Dioclea reflexa (Hook.f.) C.Wright is a beneficial species of tropical plant that grows from seeds; other names, including marble vine, sea beans, and horse-eye, also refer to it. *Agba-arin* is the name given to *D. reflexa* by the Yoruba people of South-Western Nigeria. At the same time, it is regarded as *ukpo* and *ebba* by the Igbo people of South-Eastern Nigeria (Ajatta et al. 2019). The *D. reflexa* is found in three different varieties "dark brown, light brown, and black"; it is a member of the family Leguminosae, sub-family Papillionoideae, a dicotyledonous plant and an angiosperm (Akinyede et al. 2017). The plant is a perennial plant; the seeds are in pods, each containing as many as eight seeds (Dutta 2018). The seeds are well known in the central and eastern parts of Nigeria and central African countries and have been used as traditional soup thickeners and as a rheology modifier in processed foods (Iliemene and Atawodi 2014). The importance of two notable sterols found in the seeds of *D. reflexa* has been reported; Stigmasterol has been described as a promising molecule that can be used in the development of drugs against several types of cancers, including breast, gastric, colon, prostate, and ovarian (Bakrim et al. 2022) while Taraxasterol has been described to be an excellent anti-inflammatory, anti-proliferating and antioxidative agent (Jiao et al. 2022; Movahhed et al. 2023). The research done by Balapangu et al. (2021) has also revealed that the acidic eluate of *D. reflexa* seed metabolites exhibits remarkable in vitro anti-proliferative effects on Michigan Cancer Foundation-7 (MCF-7) cells, which are used to model breast cancer.

This current study was designed to examine the phytochemical contents of *D. reflexa* seeds ethanolic extract as well as the extract's dose-dependent effects on oxidative status, lipid profile, and peroxidative status of the liver and kidney in adult male Wistar rats. The findings derived from this current study will provide insight into the antimicrobial properties, free radical scavenging activities, and antioxidant effect of *D. reflexa* seeds, which may suggest its therapeutic effect on liver and kidney health.

MATERIALS AND METHODS

Chemicals and reagents

Every reagent and chemical utilized for this research was pure and analytical grade. Gallic acid, hydrogen peroxide, ascorbic acid, and DPPH were purchased from Sigma–Aldrich, Gillingham, United Kingdom. Commercial assay kits for antioxidant status—GR, GPx activity, and Lipid parameters assays—were procured from Randox Laboratories Limited, Crumlin, United Kingdom.

Collection of plant samples, authentication, and extraction

Viable *D. reflexa* seeds were collected from four locations in Ogun state, Nigeria. The identification of the plant as *D. reflexa* was done at the Plant Science Department on the main campus of Olabisi Onabanjo University, Ago-Iwoye, Nigeria, after which both the plant and the seeds samples were kept in the herbarium. Afterward, the seeds were divided into smaller pieces, cleaned, and air-dried for two weeks at room temperature. After being ground into a coarse powder, 300 g of the dry seeds were soaked in 1000 mL of 99.7% ethanol for five days for sufficient extraction. At the end of the five days, the soaked seed sample was decanted, followed by filtration using a wool funnel to ensure that the filtrate does not contain any impurity that may compromise the usage of pure extract for subsequent analyses. Evaporation of the solvent from the *D. reflexa* seeds sample filtrate was achieved by using RotoVap 110. Further concentration to dryness was obtained on the slurry after evaporation at 40°C through a rotary evaporator. After that, lyophilization of the concentrated product into a powdery substance was done, weighed, and kept in a dry and air-tight container.

Animal care and experimental design

Guidelines for the care and use of laboratory animals were followed in conducting this research, and ethical approval was secured from "The Animal and Human Health Ethics Committee of Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Ago-Iwoye." A week was spent on the acclimatization of thirty-two (32) adult male Wistar rats, weighing between 160 and 180 g, obtained from the central animal house of INRAT "Institute of Advanced Medical Research Training" at UCH "University College Hospital" Ibadan, Nigeria. The rats were then housed in the animal house of the Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ogun state. Each animal was kept in a metallic cage in a well-ventilated room with a 12:12 hour light and dark cycle every 24 hours while maintaining a temperature of about 25ºC. They were fed regular pelletized rat chow throughout the trial and unlimited water. The rats were randomly divided into four treatment groups, consisting of eight (8) rats each: group CNTL, which received diluted ethanol as control; group DIO-5, which received 5 mg/kg of ethanolic extract of *D. reflexa* seeds; group DIO-10, which received 10 mg/kg of ethanolic extract of *D. reflexa* seeds; and group DIO-15, which received 15 mg/kg of ethanolic extract of *D. reflexa* seeds. Every day for fourteen (14) days, a single dose was administered by oral gavage. Following a twelve-hour fast and diethyl ether anesthesia, rats in each group were sacrificed after fourteen (14) days of treatment. The rats were assessed according to the effects of various extract concentrations after blood samples were drawn from the inferior vena cava of their hearts into plain centrifuge tubes. The serum was then produced by centrifugation. After being removed, the kidney and liver were dried, weighed, and cleaned in an ice-cold potassium chloride (KCl) solution (1.15%). To extract the post-mitochondrial supernatant fraction, liver and kidney samples were homogenized in four volumes of 5 mM phosphate buffer "pH 7.4". The samples were spun at 10,000 x g using a centrifuge to obtain supernatants kept for further analysis at -80°C.

Biochemical assays

Phytochemistry

The qualitative assay for the phytochemical contents of *D. reflexa* seeds ethanolic extract was conducted using different chemical procedures described by Egbuna et al. (2018).

Assessment of the presence of alkaloids

The presence of alkaloids was assessed by combining 1g of the extract with 5 mL of ethanol and 50:50 diluted hydrochloric acid in a test tube. The resultant mixture was then heated to a boiling point for ten minutes. Afterward, the solution was filtered; potassium mercuric iodide, "also known as Mayer's reagent," was added to the filtrate. Alkaloids were present when a yellow-colored precipitate formed.

Assessment of the presence of flavonoids

Exactly 0.5 g of the extract was heated for three minutes in a steam bath with 10 mL of ethyl acetate added. After the mixture was filtered, 1 mL of diluted ammonia solution was mixed with 4 mL of the filtrate. The observation of yellow coloring indicated a positive test result for flavonoids.

Assessment of the presence of tannins

In a test tube, 1 g of the extract was heated with 10 mL of distilled water, and the liquid was subsequently filtered. After adding two to three drops of 5% ferric chloride ($FeCl_3$), the presence of tannins was determined by looking for a brownish-green-black or blue-black hue.

Assessment of the presence of phenols

Exactly 1 g of the extract was dissolved with 5 mL of distilled water, and 2 mL of a 1% gelatin solution comprising 10% sodium chloride was added. The formation of a white precipitate revealed the presence of phenolic compounds.

Assessment of the presence of saponins

Furthermore, 5 mL of distilled water was added to a test tube containing 0.5 g of the extract. After 15 minutes of vigorous shaking, a 1 cm^3 layer of foam was created, indicating the presence of saponins.

Assessment of the presence of steroids

Therefore, 0.6 g of the extract and 5 mL of chloroform were combined, thoroughly agitated, and filtered in a test tube. The test tube was tilted 45 degrees, and 2 mL of concentrated H_2SO_4 was gradually introduced by the side. A color shift was noticed; the acid layer had green fluorescence, indicating the presence of steroids, whereas the chloroform layer displayed a red-to-blue tint.

Assessment of the presence of glycosides

Exactly 2 mL of distilled water was used to dissolve 0.1g of the extract. The extract mixture was supplemented with 3 g of chloroform and 1 mL of a 10% ammonium solution. The development of a pink hue signified the existence of glycoside.

Assessment of the presence of terpenoids

0.5 g of the extract was diluted in 3 mL of distilled water and this was combined with 2 mL of chloroform. After carefully adding 3 mL of concentrated H_2SO_4, a layer of reddish-brown color appeared at the interface, suggesting the presence of terpenoids.

Assays for in vitro antioxidant defense activities

Three distinct chemical methods: the hydroxyl (OH^-) radical scavenging test, FRAP, the ferric reducing antioxidant potential assay, and the DPPH 2,2-Diphenyl-1-picrylhydrazyl free radical elimination potential were followed to estimate the in vitro antioxidant activity of the ethanolic extract of *D. reflexa* seeds. A total phenolic test was also performed to ascertain the extract's capacity for scavenging free radicals. Plant-based phenolic acids are vital human dietary components that exhibit tremendous antioxidant activity, among other health benefits.

Determination of DPPH free radical scavenging activity

Using 2,2-Diphenyl-1-picrylhydrazyl (DPPH), the antioxidant activity of the ethanolic extract of *D. reflexa* seeds was measured for free radical scavenging, as per Burits and Bucar (2000) protocol. A 0.004% DPPH-ethanol solution was added to 50 µL of extracts at varying concentrations (10-50%), and the resulting mixture underwent incubation for 30 minutes at room temperature. The absorbance value was measured to Quercetin at 517 nm using a UV-visible spectrophotometer. Thus, the formula indicated below was used to estimate the inhibition rate (I%) on the DPPH free radical:

Inhibition % (I%) = {(A blank- A sample)÷A blank} × 100

Where:

A blank: Absorbance of the control reaction

A sample: Absorbance of the test compounds, Quercetin: Standard,

Reference (blank)

Determination of hydroxyl radical scavenging activity

The potential effect of *D. reflexa* seeds ethanolic extract on scavenging hydroxyl free radicals was investigated using the method outlined by Kunchandy and Rao (1990), with slight changes. A medium comprising KH_2PO_4-KOH buffer (20 mM, pH 7.4), H_2O_2 (1.0 mM), 2-deoxy-2-ribose (2.8 mM), $FeCl_3$ (100 µM), ascorbic acid (100 µM) and EDTA (100 µM) was combined with plant extract at varying concentrations to make a total volume of 1 mL; succeeded by incubation for 1 hour at 37°C. Next, to develop the color, 1 mL of 2.8% trichloroacetic acid (TCA) and 1 mL of 1% aqueous thiobarbituric acid (TBA) were added to the mixture. Afterward, 0.5 mL of the reaction mixture was heated at 90°C for 15 minutes. Following cooling, the absorbance at 532 nm was measured compared to a suitable blank solution. The following formula was applied for computing the hydroxyl radical scavenging ability (%), and ascorbic acid was used as a benchmark:

[(A absorbance of blank—A absorbance of sample) / A absorbance of blank] ×100

Determination of Ferric Reducing Antioxidant Potential (FRAP)

The method developed by Oboh and Omoregie (2011) was used to calculate the FRAP (Ferric-Reducing Antioxidant Potential) with slight changes. Exactly 250 µL of 1% potassium ferricyanide was added to the mixture of 50 µL of the extract and 450 µL of 200 mM sodium phosphate buffer (pH 6.6). For twenty minutes, the mixture underwent incubation at 50°C. Thereafter, 10% trichloroacetic acid (250 µL) was introduced, after which the mixture was centrifuged at 400 x g for ten minutes. Then 500 µL of 0.1% $FeCl_3$ was combined with 10 µL of the supernatant, and using spectrophotometry, the absorbance value of the mixture was determined at 700 nm. Every test was conducted three times. A rise in the reaction mixture's absorbance suggested that the plant samples' reducing power had increased. The standard was ascorbic acid.

Determination of Total Phenolic Content (TPC)

As per Singleton et al. (1999), the Folin-Ciocalteu assay was estimated to ascertain the phenolic constituent of *D. reflexa* seeds ethanolic extract. This technique provides reducing capability based on electrons, which is measured in terms of phenolic content. The solvent used for extraction affects both the yield and the total phenolic content of *D. reflexa* seeds ethanolic extract. Gallic acid was used for external calibration at 0.00, 0.25, 0.50, 0.75, and 1 mM concentrations. Then, 2.0 mL of solution A (mixture of 0.1 mL of sodium and potassium tartrate, 0.1 mL of $CuSO_4$, 10 mL of 2% Na_2CO_3) and 200 µL of extracts (10 mg/mL) were poured. After 4 minutes, 0.4 mL of 0.5 M sodium hydroxide solution was added. After another 10 minutes, there was an addition of 0.2 mL of the Folin-Ciocalteu reagent (1:1 v/v with water). The solution was left to stand for thirty minutes, after which its absorbance value was taken at 750 nm using a UV-Vis spectrophotometer. Following the adoption of the Gallic acid calibration curve, total phenolic content was estimated as mM Gallic acid equivalent (mM GAE).

Assays for in vivo antioxidant defense activities

Catalase assay

The catalase activity (CAT) in the kidney, liver, and serum was estimated using a slightly modified method attributed to Hadwan (2018). Catalase activity was determined as a function of micromole H_2O_2 broken down per milliliter (mL) of serum or milligrams (mg) of protein (organs) per minute.

Superoxide dismutase assay

The Del Maestro and McDonald (1987) method was adopted to ascertain the activity of superoxide dismutase (SOD) in the serum as well as liver and kidney tissues. The enzyme quantity that suppresses the oxidative conversion of epinephrine by 50%, or 1 U per milligram of protein in the serum or organs, is what is known as the SOD activity. Thus, SOD activity was evaluated as superoxide anion reduced per milliliter (serum) or mg protein per minute.

Glutathione Peroxidase (GP_X) activity assay

Following the usage of commercial kits for GPx assay (Ran-Sel from Randox, UK), the activities of GPx in the serum, liver, and kidney tissues were measured at 340 nm as NADPH oxidized on a Cobas Mira-Plus analyzer of biological fluids, a product of Roche. One micromole of NADPH oxidized per milliliter (serum) or milligram (organs) of protein per minute was the definition of an activity unit.

Glutathione Reductase (GR) activity assay

A commercial kit measured GR enzyme activity in serum, liver, and kidney tissues (Randox, UK). NADPH consumption was used as a spectrophotometric method to evaluate the enzyme activity at 340 nm. One micromole of NADPH oxidized per milliliter (serum) or milligram (organs) of protein per minute was the definition of an activity unit.

Glutathione assay

The method attributed to Tipple and Rogers (2012) was used to conduct the glutathione (GSH) assay in the serum, liver, and kidney. The glutathione concentrations were calculated and expressed as micromoles per milliliter of serum or milligram of protein (organs).

Assessment of lipid parameters

The concentrations of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) were determined in the serum using the cholesterol oxidase, glycerol-3-phosphate oxidase, and HDL Cholesterol-Direct Clearance methods, respectively. The serum's low-density lipoprotein (LDL) concentration was extrapolated using the formula in Friedewald et al. (1972). The assays were performed using commercial kits acquired from Randox Laboratories Ltd. (Crumlin, UK) following the instructions provided by the manufacturer.

Liver and kidney lipid peroxidation

The extent of lipid peroxidation in both the kidney and the liver tissue homogenates was determined spectrophotometrically according to the method of Khoubnasabjafari et al. (2015) as regards the measurement of TBARS thiobarbituric acid-reactive substance and the subsequent formation of malondialdehyde (MDA) per mg protein.

Statistical analyses

Data are displayed as mean ± SEM "standard error of means" and shown as bar charts "with SEM as the error bars." Statistical comparisons among the treatment and control groups were determined using one-way or two-way Analysis of Variance (ANOVA) followed by Dunnette's multiple comparison test. Statistical significance was set at values with $p<0.05$. GraphPad Prism software version 9.0.0 GraphPad Software, San Diego, California, USA, was used for the data representation in this study.

RESULTS AND DISCUSSION

Phytochemistry of the ethanolic extract of *D. reflexa* seeds

The qualitative phytochemical screening of *D. reflexa* seeds ethanolic extract was performed and the result is presented in Table 1.

In vitro antioxidant activities and total Phenolic content of ethanolic extract of *D. reflexa* seeds

Table 2 presents the results of the in vitro antioxidant activities and total phenolic content of the ethanolic extract of *D. reflexa* seeds determined in this study.

Effects of ethanolic extract of *D. reflexa* seeds on antioxidant enzyme activities of Wistar rats

Upon administering varying amounts of ethanolic extract of *D. reflexa* seeds, Wistar rats' blood, liver, and kidney showed noticeably elevated antioxidant enzyme activities. The activities of Catalase (Figure 1.A), Superoxide dismutase (Figure 1.B), Glutathione reductase (Figure 1.C), and Glutathione peroxidase (Figure 1.D) were significantly higher in Wistar rats treated with *D. reflexa* relative to the control rats.

Effects of ethanolic extract of *D. reflexa* seeds on reduced glutathione concentration in Wistar rats

Treatment with ethanolic extract of *D. reflexa* seeds significantly elevated GSH levels in male Wistar rats. A marked increase in GSH levels in Wistar rats was observed in the serum, kidney, and liver after administering 10 and 15 mg/kg of *D. reflexa* seed extract via oral route compared to the corresponding controls (Figure 2).

The response of lipid profile status to ethanolic extract of *D. reflexa* seeds in Wistar rats.

The serum total cholesterol level was significantly reduced by 10 and 15 mg/kg ethanolic extract of *D. reflexa* seeds; Low-density lipoprotein level was significantly reduced by 5 and 10 mg/kg ethanolic extract of *D. reflexa* seeds while High-density Lipoprotein level was significantly increased by 15 mg/kg ethanolic extract of *D. reflexa* seeds relative to the corresponding control. The results are presented in mg/dL (Figure 3).

Table 1. The outcome of the qualitative phytochemical screening of ethanolic extract of *D. reflexa* seeds following diverse chemical techniques to detect a range of bioactive compounds spontaneously occurring in medicinal plants

Phytochemicals	*Dioclea reflexa* seeds
Alkaloids	++
Flavonoids	++
Tannins	+
Phenolic compounds	++
Saponins	+
Steroids	++
Glycosides	+
Terpenoids	+

Note: + indicates present, and ++ indicates highly present

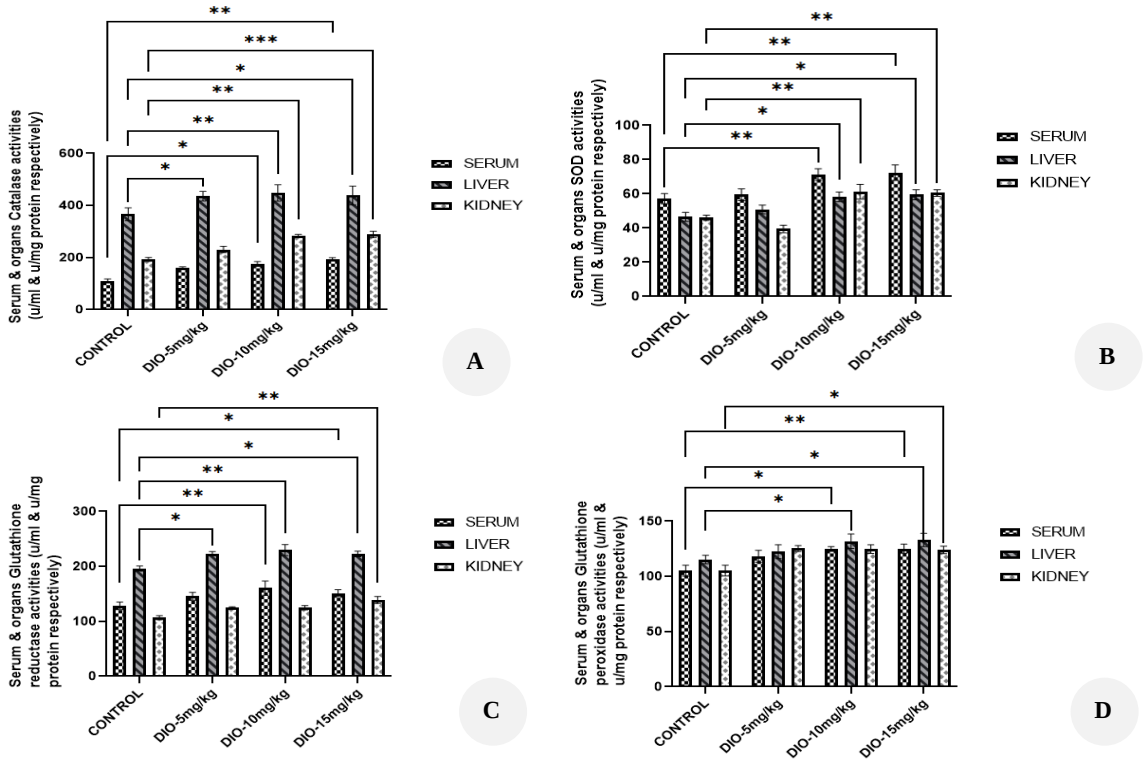

Figure 1. The effects of ethanolic extract of *D. reflexa* seeds on antioxidant enzyme (A. Catalase, B. Superoxide dismutase (SOD), C. Glutathione reductase, D. Glutathione peroxidase) in Wistar rats. Bar charts represent the activities of antioxidant enzyme in control, 5 mg/kg, 10 mg/kg, and 15 mg/kg treated rats' serum, liver, and kidney. Three asterisks indicate $p<0.005$, two indicate $p<0.01$, and one indicates $p<0.05$

Table 2. The 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl (OH^-), ferric Reducing Antioxidant Potential (FRAP) free radical scavenging activities are represented as percentages, and the total phenolic contents are expressed as Gallic acid equivalence between the *D. reflexa* seed ethanolic extract and the matching standard for comparison

Test substance	DPPH (%) ± SEM	OH^- (%) ± SEM	FRAP (%) ± SEM	Total phenolic content (mM GAE/g)
Dioclea reflexa seeds ethanolic extract	39.8±0.93[b]	22.5±1.07[b]	1.7±0.03[b]	166.9±5.43[b]
Quercetin	31.7±2/97[a]			
Ascorbic acid		18.9±0.88[a]	0.33±0.01[a]	
Gallic acid				140.3±3.60[a]

Note: Values are presented as mean ± SEM (n = 3). Values in the same column with different superscript letters (a-b) are significantly different ($p < 0.05$)

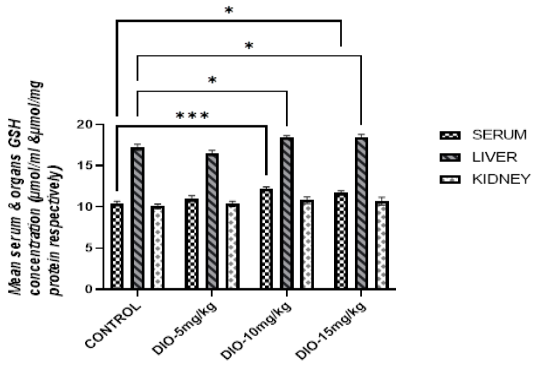

Figure 2. Effects of ethanolic extract of *D. reflexa* seeds on reduced glutathione concentration in Wistar rats. Bar charts represent reduced Glutathione (GSH) levels in the serum, liver, and kidney of Wistar rats. All results are presented as mean ± SEM. One asterisk indicates $p<0.05$, and three indicate $p<0.005$

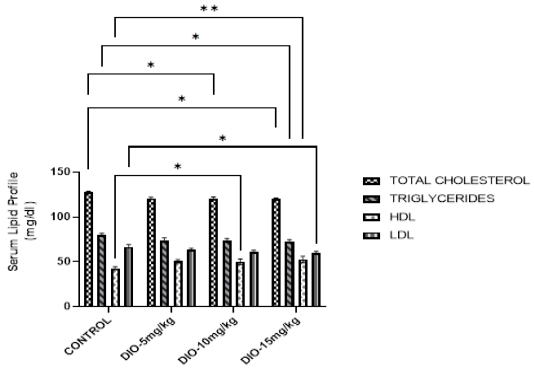

Figure 3. Effects of ethanolic extract of *D. reflexa* seeds on lipid profile status of Wistar rats. Bar charts represent the levels of total cholesterol, Triglycerides, HDL, and LDL in control, 5 mg/kg body weight, 10 mg/kg body weight, and 15 mg/kg treated rats. Two asterisks indicate $p<0.01$, and one indicates $p<0.05$

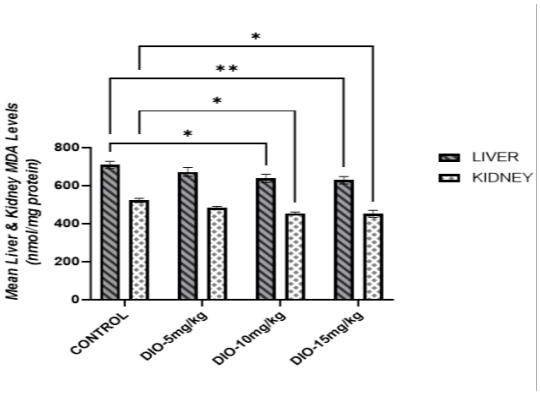

Figure 4. The effects of ethanolic extract of *D. reflexa* seeds on liver and kidney peroxidation in Wistar rats. Bar charts showing the levels of liver MDA and kidney MDA in control, 5 mg/kg bwt, 10 mg/kg, and 15 mg/kg body weight treated rats. Two asterisks indicate $p<0.01$, and one indicates $p<0.05$

The effects of ethanolic extract of *D. reflexa* seeds on kidney and liver lipid peroxidation in Wistar rats

Following the measurement of the synthesized TBARS, the levels of MDA, an index of lipid peroxidation, were significantly reduced in the liver and kidney following treatment with ethanolic extract of *D. reflexa* seeds at 10 and 15 mg/kg body weight relative to the corresponding control groups (Figure 4).

Discussion

It is impossible to overstate the value of medicinal plants as complementary therapies for managing and controlling diseases. In addition to providing food and medicine for human health, plants also aid in managing and controlling diseases (Mbah et al. 2022). Several plants have been studied to learn more about their positive effects on health and medicine by examining how they might enhance certain disease management and disease-prevention processes. In this study, the phytochemistry, in vitro antioxidant status, total phenolic content of the ethanolic extract of *D. reflexa* seeds, and subsequent outcomes of the extract on antioxidant defense, lipid profile, and the peroxidative systems of the liver and kidney investigated provide useful and logical support for using *D. reflexa* seed as an alternative disease control and management effort.

This investigation showed that the ethanolic extract of *D. reflexa* seeds included phytochemicals such as alkaloids, saponin, tannin, flavonoids, phenols, steroids, and glycosides (Table 1). According to a recently conducted research by Sharopov et al. (2018), the presence of alkaloids has been related to their therapeutic relevance in the control of numerous disorders, including Alzheimer's disease, cancer, and malaria. Sharma et al. (2018) state that these phytochemicals found in plants have antibacterial, antiviral, antiallergic, antioxidative, and antispasmodic qualities. It has been proposed that the exceptional physiological potential of tannins, phenolic compounds,

steroids, and saponins is responsible for their antibacterial properties. The use of natural products in managing and treating disease has grown rapidly, especially in underdeveloped countries. Atherosclerosis, diabetes, cancer, and other neurodegenerative diseases are some of the cardiovascular diseases being researched for prevention and treatment through Western or synthetic medicines. However, to provide accessible and affordable treatment for these conditions, developing new treatment pathways involving natural products is essential. Our research thus shows the significant documented potential therapeutic activities of the different phytochemicals present in distinct extracts from medicinal plants. Our results support the research by Oladimeji et al. (2017), who also reported the presence of important bioactive constituents in the *D. reflexa* plant.

Antioxidant properties of natural products have been demonstrated in several in vitro studies on medicinal plants (Ojha and Jain 2021; Adegbesan et al. 2024). This characteristic can help identify significant antioxidant pharmaceutical compounds for managing and treating various health issues. Low hydrogen peroxide (H_2O_2) concentrations exist naturally in the human body, the air, plants, water, food, and microbes. It disintegrates fast into oxygen (O_2) and water (H_2O), and it may also produce hydroxyl radicals (OH^-), which can cause DNA damage and thereafter initiate lipid peroxidation. *D. reflexa* seed ethanolic extract shows notable DPPH radical scavenging activity (39.8±0.93%) and considerable Ferric Reducing Antioxidant Potential (FRAP) (1.7±0.03%); it also successfully scavenges hydroxyl radicals (22.5±1.07%) (Table 2). Compared to Gallic acid, the total phenolic content was significantly higher (166.9±5.43 GAE/g) (Table 2). Our study's in vitro antioxidant results support the outcome of Atawodi and Iliemene (2014) work, who found that when exposed to acute and long-term toxicological challenges, the methanolic extract of *D. reflexa* seeds protects the blood and kidney against oxidative damage and its associated complications related ailments. Phenolic compounds are considered extraordinary and significant antioxidants that occur naturally in several plant species. Research has revealed their relationship with plant defense responses. Our observations regarding the presence and quantification of phenol recorded in this study indicate that *D. reflexa* seed extract has antioxidant potential due to the presence of phenol in the extract.

How the ethanolic extract of *D. reflexa* seeds affected the levels of antioxidant enzymes in the blood, liver, and kidney of Wistar rats, including catalase, superoxide dismutase (SOD), glutathione reductase (GR), and glutathione peroxidase (GPx) were also delved into. After administering varying doses of ethanolic *D. reflexa* seed extract, Wistar rats' blood, liver, and kidney showed noticeably elevated antioxidant enzyme activity. Overall, Wistar rats treated with *D. reflexa* showed considerably higher activities of Catalase (Figure 1.A), SOD (Figure 1.B), GR (Figure 1.C), and GPx (Figure 1.D) than the control rats. The marked increase in antioxidant enzyme activity in rats treated with *D. reflexa* may make this plant a candidate for use as a replacement for potentially toxic synthetic antioxidant analogs. Our findings regarding antioxidant enzyme activity corroborate the research by Iliemene and Atawodi (2014), who reported that the seeds of *D. reflexa* possess the ability to boost the activities of antioxidant enzymes, including catalase and SOD. Bakrim et al. (2022) also reported the antioxidative properties of stigmasterol as well as other properties, including being a molecule with anticancer, anti-osteoarthritis, antidiabetic, antiparasitic, anti-inflammatory, antibacterial, antifungal, and neuroprotective properties. Consequently, it can be used in the development of therapeutic drugs against several cancer types, including colon, breast, gastric, and ovarian. Jiao et al. (2022), also revealed that Taraxasterol found in the seed of *D. reflexa* possesses an outstanding antioxidative, anti-inflammatory, and chemo-preventive property against chemical carcinogenesis. The study's observation of the ethanolic extract of *D. reflexa* seeds' antioxidative action implies that these seeds may have potential pharmaceutical applications in disease management.

In this study, a significant increase ($p<0.05$) in liver glutathione (GSH) levels was observed in rats treated with 10 and 15 mg/kg ethanolic seed extract of *D. reflexa* (Figure 2) compared to the control rats. A similar effect was also observed in the serum of the treated rats following 10 and 15 mg/kg ethanolic seed extract of *D. reflexa*. Increased GSH level indicates that the seeds of *D. reflexa* are a potential antioxidant that may shield the liver and blood from the damaging effects of oxidative stress and other toxicological conditions. These findings are supported by the research of Atawodi and Iliemene, who reported that *D. reflexa* seeds are rich in substances that have the potential to protect the kidneys and blood from oxidative damage and other related diseases (Atawodi and Iliemene 2014; Iliemene and Atawodi 2014, 2023).

Our study examined the impact of ethanolic extract of *D. reflexa* seeds on the lipid profile status of male Wistar rats. The findings (Figure 3) showed that the treated Wistar rats at 10 mg/kg and 15 mg/kg had significantly higher levels of HDL in comparison to the control rats; the treated Wistar rats at 15 mg/kg had significantly lower levels of LDL in comparison to the control rats; both the 10 mg/kg and 15 mg/kg treated Wistar rats had significantly lower levels of total cholesterol in comparison to the control rats; additionally, the level of triglycerides was significantly lower in the 15 mg/kg treated Wistar rats than the control rats. Although ethanolic extract of *D. reflexa* seeds has been shown to have a decreasing effect on the levels of both triglycerides and total cholesterol, high levels of both can pose major health hazards. Individuals with established coronary heart disease have been found to have an all-cause mortality rate that is correlated with high triglycerides (Klempfner et al. 2016); hence, administering *D. reflexa* seed extract to these individuals may lower their death rate. Rats treated with the ethanolic extract likewise showed reduced levels of LDL and a concurrently increased level of HDL. According to a recent study, HDL and LDL are related to wound healing. Chen et al. (2022) showed that treatment with HDL cholesterol restored the substantial connection between lower HDL cholesterol

levels and poorer wound healing. This implies that, given that low HDL cholesterol is a typical hallmark of diabetes, the ethanolic seed extract of *D. reflexa* may be a viable treatment to promote wound healing, particularly in cases of diabetes. Our findings on lipid profile assessment are supported by the research that found phytosterols in the *D. reflexa* seed methanolic extract. Additionally, it has been noted that these phytosterols can decrease cholesterol and Low-Density Lipoprotein (LDL), preventing the buildup of cholesterol within the body. This suggests that the phytosterol constituent of the *D. reflexa* seeds may be linked with the observed modulatory effects of the seeds on the lipid profile status of Wistar rats treated with this extract. These findings on the impact of *D. reflexa* seed ethanolic extract on the lipid profile status of Wistar rats imply that the seeds play a modulatory role in lipid metabolism and subsequently exert a beneficial impact on lipid regulation to enhance human health.

Our findings on the impact of *D. reflexa* seeds ethanolic extract on liver and kidney peroxidation in Wistar rats (Figure 4) showed a noteworthy decrease in liver and kidney MDA levels after administering 10 and 15 mg/kg of the ethanolic seed extract. This implies that the extract may treat renal and liver disorders. The observed outcome in the liver tissues of Wistar rats administered with the seed extract aligns with the findings of Iliemene and Atawodi (2014), who documented the hepatoprotective effect of methanolic *D. reflexa* seed extract in rats after liver damage. Our findings regarding the decreased levels of MDA in the kidney tissues of rats treated with ethanolic seed extract of *D. reflexa* are further supported by a study conducted in 2014 by Atawodi and Iliemene (2014), which found that the methanolic extract of the seeds of this plant protects the blood and the kidney from oxidative damage and other related injuries due to underlying toxicological damage.

In conclusion, our investigation into the phytochemical content of the ethanolic seed extract of *D. reflexa* produced some interesting results, including the presence of substances (such as tannins, saponins, phenolic compounds, and steroids) with possible antibacterial properties. The results of this study also indicate that *D. reflexa* seed has an exceptional ability to scavenge free radicals and reduce oxidative stress; additionally, it positively modulates HDL levels and may facilitate wound healing, particularly in cases of diabetes, due to its modulatory effect on lipid profile status. In addition, due to its antioxidant qualities, *D. reflexa* seeds may help to manage and treat liver and kidney problems. This study offers justification for more investigation into the molecular mechanisms behind the interactions and modifications of metabolic processes by substances found in seed extracts of *D. reflexa*.

ACKNOWLEDGEMENTS

We greatly appreciate Prof. Oladipo Ademuyiwa of the Department of Biochemistry at the Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria, for his efforts and support in making this study a success. The authors would also like to extend their appreciation to the animal house staff at the Faculty of Basic Medical Sciences, Olabisi Onabanjo University in Ogun State, Nigeria, for their animal handling skills and efforts in animal management.

REFERENCES

Adegbesan BO, Ezima EN, Hassan BO, Kehinde JO, Adewale AA, Osonuga IO. Olalekan SO. 2024. Evaluation of in vitro antioxidant activities, total phenolic and elemental contents of common herbs and spices (*Moringa oleifera* leaves, *Allium sativum* (Garlic), and *Momordica charantia* (ejinrin) leaves) in South-West Nigeria. Pure Appl Chem 2024. DOI: 10.1515/pac-2023-1128.

Ajatta MA, Akinola SA, Otolowo DT, Awolu OO, Omoba OS, Osundahunsi, OF. 2019. Effect of roasting on the phytochemical properties of three varieties of marble vine (*Dioclea reflexa*) using Response Surface Methodology. Prev Nutr Food Sci 24 (4): 468-477. DOI: 10.3746/pnf.2019.24.4.468.

Akinyede AI, Girgih AT, Osundahunsi OF, Fagbemi TN, Aluko RE. 2017. Effect of membrane processing on amino acid composition and antioxidant properties of marble vine seed (*Dioclea reflexa*) protein hydroxylate. J Food Process Preserv 41 (3): e12917. DOI: 10.1111/jfpp.12917.

Anwar MA, Al Disi SS, Eid AH. 2016. Anti-hypertensive herbs and their mechanisms of action: Part II. Front Pharmacol 7: 172328. DOI: 10.3389/fphar.2016.00050.

Atawodi SE, Iliemene U. 2014. Effects of methanolic extract of *Dioclea reflexa* Hook F seed on some haematological and kidney function parameters in rats following single or repeated carbon tetrachloride intoxication. Br Biotechnol J 4: 1253-1262. DOI: 10.9734/BBJ/2014/13836.

Bakrim S, Benkhaira N, Bourais I, Benali T, Lee LH, El Omari N, Sheikh RA, Goh KW, Ming LC, Bouyahya A. 2022. Health benefits and pharmacological properties of stigmasterol. Antioxidants 11 (10): 1912. DOI: 10.3390/antiox11101912.

Balapangu S, Nyankson E, Asimeng BO, Asiamah R, Arthur PK, Tiburu, EK. 2021. Capturing *Dioclea reflexa* seed bioactive on halloysite nanotubes and pH-dependent release of cargo against breast (MCF-7). Separations 8 (3): 26. DOI: 10.3390/separations8030026.

Burits M, Bucar F. 2000. Antioxidant activity of *Nigella sativa* essential oil. Phytother Res 14 (5): 323-328. DOI: 10.1002/1099-1573(200008)14:5%3C323::aid-ptr621%3E3.0.co;2-q.

Chen L, Ma W, Chen D, Wang C, Gao Y, Ran X. 2022. Association of high-density lipoprotein cholesterol and wound healing in patients with diabetic foot ulcers. Chin Med J 135: 110-112. DOI: 10.1097/CM9.0000000000001544.

Chopra B, Dhingra AK. 2021. Natural products: A lead for drug discovery and development. Phytother Res 35 (9): 4660-4702. DOI: 10.1002/ptr.7099.

Del Maestro R, McDonald W. 1987. Distribution of superoxide dismutase, glutathione peroxidase, and catalase in developing rat brain. Mech Ageing Dev 41 (1-2): 29-38. DOI: 10.1016/0047-6374(87)90051-0.

Dutta AC. 2018. Botany for Degree Students. Oxford University Press. New Delhi.

Egbuna C, Ifemeje JC, Maduako MC, Tijjani H, Udedi SC, Nwaka AC, Ifemeje MO. 2018. Phytochemical test methods: qualitative, quantitative, and proximate analysis. In: Egbuna C,Ifemeje JC, Udedi SC, Kumar S (eds). Phytochemistry. Apple Academic Press, Florida. DOI: 10.1201/9780429426223-15.

Friedewald WT, Levy RI, Fredrickson DS. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18 (6): 499-502. DOI: 10.1093/clinchem/18.6.499.

Hadwan MH. 2018. Simple spectrophotometric assay for measuring catalase activity in biological tissues. BMC Biochem 19: 7. DOI: 10.1186/s12858-018-0097-5.

Iliemene UD, Atawodi SE. 2014. In vivo antioxidant and hepatoprotective effects of methanolic extract of *Dioclea reflexa* seed in rats following acute or chronic liver injury. Bangladesh J Pharmacol 9: 112-117. DOI: 10.3329/bjp.v9i1.17452.

Iliemene UD, Atawodi SE. 2023. Mitigating effect of dietary *Dioclea reflexa* (Hook F) seed inclusion in experimental colon carcinogenesis. J Food Biochem 2023: 2823143. DOI: 10.1155/2023/2823143.

Jiao F, Tan Z, Yu Z, Zhou B, Meng L, Shi X. 2022. The phytochemical and pharmacological profile of taraxasterol. Front Pharmacol 13: 927365. DOI: 10.3389/fphar.2022.927365.

Jimenez-Garcia SN, Vazquez-Cruz MA, Garcia-Mier L, Contreras-Medina LM, Guevara-González RG, Garcia-Trejo JF. Feregrino-Perez AA. 2018. Phytochemical and pharmacological properties of secondary metabolites in berries. In: Holban AM, Grumezescu AM (eds). Therapeutic Foods. Academic Press, London. DOI: 10.1016/b978-0-12-811517-6.00013-1.

Khoubnasabjafari M, Ansarin K, Jouyban A. 2015. Critical review of Malondialdehyde analysis in biological samples. Curr Pharm Anal 12 (1): 4-17. DOI: 10.2174/1573412911666150505185343.

Klempfner R, Erez A, Sagit B, Goldenberg I, Fisman E, Kopel E, Shlomo N, Israel A, Tenenbaum A. 2016. Elevated Triglyceride level is independently associated with increased all-cause mortality in patients with established coronary heart disease. Circ Cardiovasc Qual Outcomes 9: 100-108. DOI: 10.1161/circoutcomes.115.002104.

Kunchandy E, Rao MNA. 1990. Oxygen radical scavenging activity of curcumin. Intl J Pharm 58 (3): 237-240. DOI: 10.1016/0378-5173(90)90201-e.

Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, Gargiulo G, Testa G, Cacciatore F, Bonaduce D, Abete P. 2018. Oxidative stress, aging, and diseases. Clin Interv Aging 13: 757-772. DOI: 10.2147/cia.s158513.

Mbah CC, Samali A, Aboh MI, Ogbonna JI, Builders, PF, Attama AA, S.I. Ofoefule SI. 2022. Preliminary investigation of Dioclea reflexa seed gum as a food and potential pharmaceutical excipient. German J Pharm Biomater 1 (4): 27-37. DOI: 10.5530/gjpb.2022.4.17.

Movahhed M, Pazhouhi M, Ghaleh HEG, Kondori BJ. 2023. Anti-metastatic effect of taraxasterol on prostate cancer cell lines. Res Pharm Sci 18 (4): 439-448. DOI: 10.4103/1735-5362.378090.

Noor F, Tahir ul Qamar M, Ashfaq UA, Albutti A, Alwashmi AS, Aljasir MA. 2022. Network pharmacology approach for medicinal plants: review and assessment. Pharmaceuticals 15 (5): 572. DOI: 10.3390/ph15050572.

Oboh HA, Omoregie IP. 2011. Total phenolics and antioxidant capacity of some Nigerian beverages. Nigerian J Basic Appl Sci 19 (1): 68-75. DOI: 10.4314/njbas.v19i1.69346.

Ojha DK, Jain AP. 2021. Phytochemical screening, estimation of total phenolic, flavonoid content, and estimation of antioxidant activity of hydroalcoholic extract of *Vitex negundo*, *Vitex trifolia*, and *Vitex parviflora*. J Adv Sci Res 12 (04): 268-271. DOI: 10.55218/jasr.202112436.

Oladimeji A, Oladosu I, Alli S. 2017. P22 Discovery of new bioactive compounds from (*Dioclea reflexa* Hook F): Effective constituents of a traditional herbal medicine. Biochem Pharmacol 139: 132. DOI: 10.1016/j.bcp.2017.06.023.

Shaito A, Thuan DTB, Phu HT, Nguyen THD, Hasan H, Halabi S, Abdelhady S, Nasrallah GK, Eid AH, Pintus G. 2020. Herbal medicine for cardiovascular diseases: Efficacy, mechanisms, and safety. Front Pharmacol 11: 422. DOI: 10.3389/fphar.2020.00422.

Sharma BR, Kumar V, Gat Y, Kumar N, Parashar A, Pinakin D J. 2018. Microbial maceration: A sustainable approach for phytochemical extraction. 3 Biotech 8: 401. DOI: 10.1007/s13205-018-1423-8.

Sharopov F, Valiev, A, Gulmurodov I. Sobeh, M, Satyal, P Wink M. 2018. Alkaloid content, antioxidant, and cytotoxic activities of various parts of *Papaver somniferum*. Pharm Chem J 52: 459-463. DOI: 10.1007/s11094-018-1839-9.

Singleton VL, Orthofer R, Lamuela-Raventós RM. 1999. [14] Analysis of total phenols and other oxidation substrates and antioxidants using folin-ciocalteu reagent. Methods Enzymol 299: 152-178. DOI: 10.1016/s0076-6879(99)99017-1.

Tipple TE, Rogers LK. 2012. Methods for the determination of plasma or tissue glutathione levels. In: Harris C, Hansen JM (eds). Developmental Toxicology: Methods and Protocols. Springer, Humana Totowa, NJ. DOI: 10.1007/978-1-61779-867- 2_20.

Ye M, Moon J,Yang J, Lim HH, Bin Hong S, Shim I. *et al*. 2015. The standardized *Lycium chinense* fruit extract protects against Alzheimer's disease in 3xTg-AD mice. J Ethnopharmacol 172: 85-90. DOI: 10.1016/j.jep.2015.06.026.