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Evaluation of the *in vitro* Antioxidant Activities of Leaves Extracts of *Persea americana* and *Ficus exasperata* Collected from Akure, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SIA and OEA designed the study, wrote the protocol and supervised the work. Authors SIA, OEA and OAA carried out all laboratories work. Author SIA performed the statistical analysis. Author OAA managed the analyses of the study. Author SIA wrote the first draft of the manuscript. Author OEA managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study examined the phytochemical composition and antioxidant potentials of leaves extracts of *Persea americana* and *Ficus exasperata* obtained from Akure, Ondo state.
Study Design: Methanol and acetone were used as extracting solvents for the leaves samples. Test for alkaloids, terpenoids, cardiac glycoside, tannin, saponin, vitamin C, total flavonoids, total phenolics as well as the *in vitro* antioxidant assays such as scavenging of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radicals, chelation of ferrous ion, and inhibition of iron II (Fe^{2+}) induced lipid peroxidation were evaluated.
Results: The acetone extract of *F. exasperata* has the highest alkaloids (68.60%), total phenol (5.08 mg/g), total flavonoids (4.94 mg/1 g) and vitamin C contents (22.30 mg/g). At a concentration of 2 mg/mL, the leaves extracts were able to scavenge DPPH (above 70%), chelate ferrous ion (above 70%) and inhibit the production of malondialdehyde (below 30%). However, the positive controls, Butylated hydroxyl toluene (3,5-di-tert-butyl-4-hydroxytoluene) and Ethelenediaminetetraacetic acid, were more effective than the leaves extracts at

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similar concentration.
Conclusion: Results from this study revealed that the leaves of *Persea americana* and *Ficus exasperata* are effective in combating free radicals and their effects *in vitro*. It therefore supports the claims that the plants leaves are promising candidates in developing drugs for the treatment of radical associated ailments.

Keywords: Extracts; oxidative stress; malondialdehyde; phenolic compounds; Persea Americana; Ficus exasperata.

1. INTRODUCTION

Nature remains an inexorable source of various pharmacological agents. Novel chemical compounds with unique structural diversity found in natural products such as plants, has provided boundless opportunities for the discovery of new and important drugs [1,2].

Plants have been used traditionally from time immemorial for the prevention and treatment of many diseases [3,4]. These medicinal plants still remain an exclusive source for life saving drugs for majority of the world's population [2]. The pharmacological activities of these medicinal plants have been attributed to the presence of secondary metabolites which are known to play a major role in the adaptation of plants to their environment [5].

Pathological conditions such as diabetes, atherosclerosis, ischemia, asthma, arthritis aging, immunosuppression and neurodegeneration as a result of degenerative effects of free radicals to body cells and tissues, has led to sourcing for antioxidants from natural sources [6]. Combating these free radicals with synthetic antioxidants such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) has raised serious concerns due to their various adverse effects such as toxicity and carcinogenicity [7]. However, improvement of the body antioxidant status through the consumption of natural fruits and vegetables has reportedly reduced the morbidity and mortality of degenerative diseases [8]. The natural antioxidants found in these plants are considered safe and with less adverse effects. These plants are known to contain bioactives such as phenolic compounds, tannins, vitamin E, vitamin C, and carotenoids that have been reported in several studies to be powerful free radical scavengers [9,10].

Ficus exasperata also known as sand paper tree belong to the family Moraceae with approximately 1000 species is wide spread in tropical and sub-tropical region of the world Africa [11,12]. Leaf extract of *Ficus exasperata* has been used in African traditional medicine for the treatment of high blood pressure, rheumatism, arthritis, intestinal pains, epilepsy, bleeding and wounds as reported in several studies [13,14]. *Persea americana* Mill. (avocado) is a tree that belongs to the family Lauraceae. It is cultivated in tropical and subtropical climates around the world and have been used in traditional medicine for the treatment of various ailments such as such as osteoarthritis, inflammatory conditions, bronchitis, diabetes, stomach ache, convulsions and epilepsy [15,16,17].

Taking into consideration the reported enormous medicinal potentials of these plants, leaves of *Ficus exasperate* and *Persea Americana* collected from Akure, were screened for their ability to inhibit Fe^{2+} induced lipid peroxidation in rat's liver *in vitro*.

2. MATERIALS AND METHODS

2.1 Collection of Leaves Samples

Leaves of *Ficus exasperata* and *Persea americana* were collected from their trees near Life Spring College, Apatapiti Layout, Federal University of Technology, Akure (FUTA), Ondo State (Latitude: 7.289N, Latitude: 5.150E) and a building opposite BTO hall Ilesha garage, Akure, Ondo State (Latitude:7.3064N, Longitude: 5.12227E) respectively in the month of April, 2015. Samples of the leaves were taken to the Department of Crop, Soil and Pest, FUTA for authentication. The leaves were washed with clean water and then air dried before grinding and labelled for easy identification.

2.2 Handling and Use of Animals

The guidelines provided by the National Institute of Health (NIH) for the care and use of laboratory animals were followed. Apparently healthy albino rats that weighed between 200–230 g were purchased from the Department of Animal

production and Health, FUTA. The rats were housed in a cage with a temperature of 25 ± 2ºC, on a 12 hour light/12 hour dark cycle. They were acclimatized under these conditions for two weeks before the experiment in which the animals were maintained *ad libitum* on commercial food and water.

2.3 Preparation of Leaves Extracts

Powdered leaves (100 g) of *Persea americana* and *Ficus exasperata* were weighed separately into different containers and extracted with 1000 mL of 100% acetone and methanol. Aluminium foil paper was placed on the containers with the solution stirred continuously for 3 days. Whatman filter paper (0.45 µm) placed in a funnel was then used to filter the solution. The filtrate obtained was thereafter subjected to evaporation in a rotary evaporator (RE-52A; Union Laboratory, England) at 50ºC and 90 rpm. The dried concentrated extracts were afterwards kept in the dark at a temperature of 4ºC prior to its use for analysis.

2.4 Determination of Tannins

The method of Association of Official Analytical Chemists [AOAC] [18], with slight modifications was used in determining tannin content of the leaves extracts. An aliquot of 20 mL of 50% methanol was mixed with 0.20 g of the leaves extracts. The mixture was thereafter shaken vigorously and placed in a water bath at 80ºC for 1 hour to ensure uniform mixing. The mixture was filtered into a 100 mL volumetric flask, followed by the addition of distilled water (20mL), 17% aq. Na_2CO_3 (Sodium carbonate) (10mL) and Folin-Denis reagent (2.5mL). After thorough mixing of the solution, distilled water was added to make it up to 100 mL and then left to stand for 20 minutes. A spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215) was subsequently used to measure the absorbance of the leaves extracts as well as the standard (tannic acid) at a wavelength of 760 nm in a glass cuvette with 1 cm optical path length.

2.5 Estimation of Saponin Content of Leaves Extracts

The method of Obadoni and Ochuko [19] was employed in estimating the saponin content of the leaves extracts of *Ficus exasperata* and *Persea americana*. Powdered sample (20 g) of the leaves extracts was added to 20% aqueous ethanol (100 mL). The mixture was then placed in a shaker for 30 minutes for thorough mixing. Afterwards, samples of the leaves extracts were heated for 4 hours at 55ºC in water bath with continuous stirring. The solution was then filtered and the residue obtained was re-extracted with 20% aqueous ethanol (200 mL). The volume of the combined extracts was brought down to about 40 mL over the water bath at 90ºC. The concentrate was conveyed into a separatory funnel (250 mL capacity) and 20 mL of diethyl ether was added for extraction with the procedure repeated. The ether layer obtained was removed while 60 mL n-butanol was added to the retained aqueous layer. This was then followed up by the washing of the n-butanol extracts with 10 mL of 5% aqueous sodium chloride. The solution left was then heated in a water bath. After evaporation of the solution, the samples obtained were dried to a constant weight in the oven at 40ºC.

2.6 Determination of Alkaloids Content of the Leaves Extracts

The alkaloids content of the leaves extracts was measured quantitatively using the method of Harborne [20]. The powdered extract (5 g) was weighed into a beaker, thereafter 10% acetic acid in ethanol (200 mL) was added to it. The beaker was then covered and the solution was left to stand for 4 hours. The solution was then filtered and the filtrate concentrated to ¼ of its original volume on a water bath. Drops of concentrated ammonium hydroxide was added to the extract until complete precipitation was observed. The solution was then left to settle and the precipitates collected were washed with dilute ammonium hydroxide and then filtered. The residue obtained was thereafter dried to a constant weight.

2.7 Estimation of Steroid Content

Estimation of the steroid content of the leaves samples was achieved using the method described by Trease and Evans [21]. The powdered plant sample (2.5 g) was weighed into a beaker containing 50 mL of distilled water and vigorously mixed for 1 hour. Thereafter, an aliquot of 2 mL of the solution was washed 3 mL of 0.1 M NaOH (pH 9). This was then mixed with chloroform (2 mL) and ice cold acetic anhydride (3 mL). Two drops of concentrated H_2SO_4 was then cautiously added to the reaction mixture. With the aid of a spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab

23A, model number 23A08215) at 420 nm in a glass cuvette with 1cm optical path length the absorbance of the sample and blank solution was measured.

2.8 Determination of Cardiac Glycosides

Cardiac glycoside content in the samples was evaluated using Buljet's reagent as described by El-Olemy et al. [22]. The samples were then purified using lead acetate and disodium hydrogen phosphate (Na_2HPO_4) solution before the addition of freshly prepared Buljet's reagent (containing 95 mL aqueous picric acid + 5 mL 10% aqueous NaOH). The difference between the intensity of colours of the experimental and blank (distilled water and Buljet's reagent) samples gives the absorbance and is proportional to the concentration of the glycosides.

2.9 Determination of Total Flavonoid Content of Leaves Extracts

The method developed by Bao [23] was used in estimating the flavonoid content of the leaves extract. An aliquot of 0.2 mL of the leaf extract was added to 0.3 mL of 5% sodium Nitrate ($NaNO_3$) at zero time. Thereafter 0.6 mL of 10% aluminium chloride ($AlCl_3$) was added to the reaction mixture after 5 minutes followed by the addition of 2 mL of 1M NaOH (sodium hydroxide) after 6 minutes and 2.1 mL of distilled water was lastly added to the reaction mixture. The absorbance of the solution was then read against the reagent blank solution at 510 nm in a glass cuvette with 1 cm optical path length with the aid of a spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215). Rutin methanolic solutions were used in preparing the calibration curve at concentrations of 12.5 to 100 µg/mL. The total flavonoid content of the leaves extract was expressed as mg rutin equivalents per gram of dried leaf extract (mg RE/g).

2.10 Estimation of the Total Phenolic Content of Leaves Extracts

The method of Singleton et al. [24] was used in determining the total phenolic content of the leaves extracts. This started by coupling the extract (0.2 mL) with 2.5 mL of 10% Folin ciocalteau's reagent and 2 mL of 7.5% sodium carbonate (Na_2CO_3). Incubation of the reaction mixture at 45ºC for 40 minutes was carried out. The absorbance of the solution was read at 700 nm in a glass cuvette with 1 cm optical path length with the aid of a spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215). Standard of gallic acid was used in plotting of the calibration. The total phenolic content of the leaves was expressed as mg gallic acid equivalents per gram of dried leaf extract (mg GAE/g).

2.11 Determination of Vitamin C Content

The method developed by Benderitter et al. [25] was used to measuring the vitamin C content of the leaves extracts of *Ficus exasperata* and *Persea americana*. To an aliquot of 500 µL of a reaction mixture made up of 300 µL of an appropriate dilution of the polar extract with 100 µL 13.3% trichloroacetic acid (TCA) and water, 75 µL of DNPH (2g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg $CuSO_4.5H_2O$ in 100 mL of 5 mol L^{-1} H_2SO_4) were added. Afterwards the resulting mixture was incubated for 3 hours at 37ºC. Subsequently 0.5 mL of 65% H_2SO_4 (v/v) was added to the medium. Measurement of the absorbance at 520 nm in a glass cuvette with 1 cm optical path length was carried out using a spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215). The leaves extracts vitamin C content was measured using ascorbic acid as the reference compound and expressed as mg/g ascorbic acid equivalent.

2.12 Fe^{2+} Chelation Assay

The method of Minotti and Aust [26] slightly modified by Puntel et al. [27], was used to estimate the chelating effect on ferrous ions. To a mixture of 168 µl of 0.1 M Tris-HCl (pH 7.4), 218 µ saline and extracts (0 – 25 µL), freshly prepared 500 µM FeSO4 (150 µL) was added. The solution was incubated for 5 minutes, thereafter 13 µL of 0.25% 1, 10-phenanthroline (w/v) was added to it. At 510nm the absorbance of the solution was measured. The reference standard used was EDTA-Na_2. The ferrous ion chelating ability of the leaves extracts was then calculated with respect to the reference.

$$\text{Ferrous ion chelating ability (\%)} = \frac{\text{Absorbance of standard} - \text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ x } 100$$

2.13 DPPH (2, 2-diphenyl-1-picrylhydrazyl) Scavenging Activity

The scavenging effect of the leaves extract of *Ficus exasperata* and *Persea americana* on DPPH radical was measured using the slightly modified method [28] of Gyamfi et al. [29]. The extract (2 mL) at varying concentrations diluted to two-fold in methanol was mixed with a solution of 1.0 mL of 0.3 mm DPPH in methanol. This was followed by a vigorous shaking of the reaction mixture. It was then left to stand in the dark for 25 minutes at room temperature. This was followed by the preparation of the blank solutions with 2 mL of each test sample solution and methanol (1 mL). The negative control used for the experiment was 1 mL of 0.3 mM DPPH solution added to 2 mL of methanol. Whereas butylated hydroxytoluene (3, 5-di-tert-butyl-4-hydroxytoluene) was used as positive control. The absorbance of the resulting was measured at 518 nm in a glass cuvette with 1cm optical path length against the blank solutions with the aid of a UV-visible spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215). The inhibition of DPPH radical by the leaves extracts was evaluated using the equation:

$$\% \text{ Inhibition} = \frac{\text{Abs control} - (\text{Abs } sample - \text{Abs blank})}{\text{Abs control}} \text{x } 100$$

Where the Abs control is the absorbance of the control (DPPH without sample), the Abs sample is the absorbance of the test sample (the sample test and DPPH solution), and the Abs blank is the absorbance of the sample blank (Sample without the DPPH solution).

2.14 Estimation of Lipid Peroxidation Inhibition by Leaves Extracts

2.14.1 Preparation of rat's liver homogenate

The albino rats were killed under mild diethyl ether anaesthesia and their liver was quickly cut opened, weighed and placed on ice. Subsequently, a Teflon-glass homogenizer was used for the homogenization of the liver tissue in cold saline (1:10 w/v) with about 10-up-and-down strokes at about 1200 rev/min. Afterwards, the homogenate obtained was centrifuged at 3000 × g for 10 min to obtain a low-speed supernatant (SI) that was kept for lipid peroxidation analysis and a pellet that was disposed [30].

2.14.2 Lipid peroxidation and thiobarbibutric acid reactions

The modified method of Ohkawa et al. [31] was employed in assaying lipid peroxidation inhibitory potential of the leaves extracts. The SI fraction (100 µl) was added to a reaction mixture containing 30 µl of 0.1 M Tris-HCl buffer (pH 7.4), extract (0 – 100 µl) and 30 µl of the prooxidant (250µM freshly prepared Iron II sulphate $FeSO_4$). Afterwards, 300 µl of water was added to the mixture prior to incubation for an hour at 37ºC. The addition of 300 µl of 8.1% sodium dodecyl sulphate to the reaction mixture containing SI fraction, followed by the addition of 600 µl of acetic acid/HCl (pH 3.4) coupled with 600 µl of 0.8% thiobarbituric acid resulted into colour reaction. The resulting mixture was afterwards incubated for an hour at 100ºC. Subsequently, the absorbance of the thiobarbituric acid reactive species (TBARS) formed was measured at 532 nm in a glass cuvette with 1 cm optical path length. The amount of malondialdehyde (MDA) generated was expressed as % control using MDA standard curve (0-0.035 mM).

2.15 Statistical Analysis

All the experiments carried out in this study were in triplicates. The data obtained were analyzed using One Way Analysis of Variance (ANOVA). The means were compared using the New Duncan's Multiple Range Test (SPSS version 16) and differences were considered to be significant at P=0.05.

3. RESULTS

Result for the extraction yield of the leaves extracts displayed in Table 1 shows that methanol gave higher yield of extract (23.19% for *F. exasperata* and 9.21% for *P. americana*) than acetone (3.08% for *F. exasperata* and 5.44% for *P. americana*).

The methanol extract *Persea americana* displayed the highest concentration of saponin (14.22 mg/g) while the acetone extract of *Ficus exasperata* gave highest concentration of alkaloids (68.60%), vitamin C (22.30 mg AAE/g), total flavonoids (4.94 mg RE/g) and total phenolics (5.08 mg GAE/g). Whereas the highest concentration of terpenoids (20.60 mg/g), cardiac glycoside (13.49 mg/g) and tannin (4.42 mg/g)

was displayed by the acetone extract of *Persea americana* (Table 2).

Table 1. Yield of leaves extracts of *Ficus exasperata* and *Persea americana*

Leaves	Solvents	Yield (%)
Ficus exasperata	Methanol	23.19
	Acetone	3.08
Persea americana	Methanol	9.21
	Acetone	5.44

The ferrous chelating effect of the leaves extracts of *F. exasperata* and *P. americana* is displayed in Fig. 1. The leaves extracts displayed appreciable iron chelating activity which was higher than 50% at a concentration of 1.5 mg/mL. The activity of the extracts was concentration dependent, increasing with increasing concentration. The methanol extract of *F. exasperata* displayed the highest chelating activity (72.09%) at a concentration of 2 mg/mL. The positive control (EDTA) however displayed higher chelating activities than the extracts at all the tested concentrations (98.87% at 2 mg/mL).

The DPPH scavenging activity of the leaves extracts is displayed in Fig. 2. The extracts displayed varying and concentration dependent radical scavenging activity. The extracts displayed appreciable radical scavenging activity with the acetone extracts of *F. exasperata* and *P. americana* exhibiting better activities at all test concentrations. At a concentration of 2 mg/mL, the acetone extract of *P. americana* had the highest activity (81.14%). However, the positive control BHT displayed better and significantly different activity (98.82%) at the same concentration.

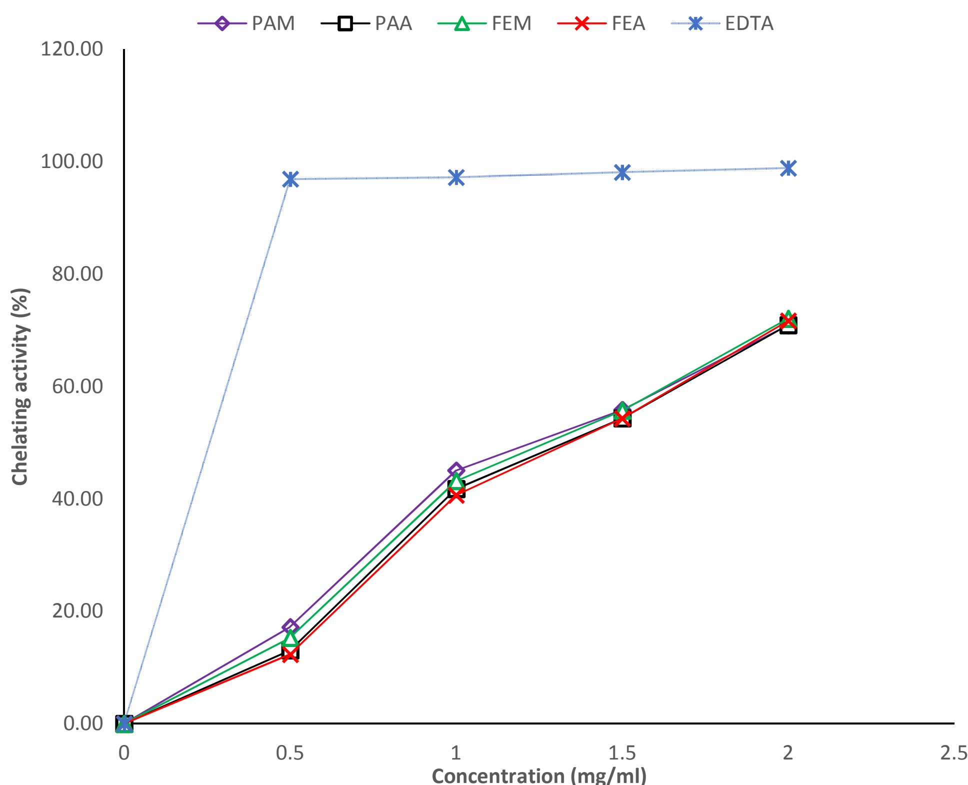

Fig. 1. Ferrous chelating activity of leaves extracts of *Ficus exasperata* and *Persea americana*

Each value is mean of triplicate results (n=3).
Keys: FEA: Acetone leaf extract of Ficus exasperata;
FEM: Methanol leaf extract of Ficus exasperata; PAA: Acetone leaf extract of Persea americana;
PAM: Methanol leaf extract of Persea Americana; EDTA: Ethelenediaminetetraacetic acid

Table 2. The Phytochemical constituents of leaves extracts of *Ficus exasperata* and *Persea Americana*

Phytochemical	Extracts			
	FEM	**FEA**	**PAM**	**PAA**
Alkaloids (%)	61.63±0.19^{b}	68.60±3.58^{c}	0.00±0.00^{a}	0.00±0.00^{a}
Terpenoids (mg/g)	10.92±0.19^{b}	15.84±3.58^{c}	11.51±0.00^{a}	20.60±0.10^{d}
Cardiac glycoside (mg/g)	11.09±0.05^{c}	8.31±0.07^{a}	9.94±0.00^{b}	13.49±0.09^{d}
Tannin (mg/g)	4.34±0.06^{c}	2.73±0.05^{a}	3.41±0.07^{b}	4.42±0.10^{c}
Saponin (mg/g)	0.00±0.00^{a}	0.00±0.00^{a}	14.22±0.11^{c}	10.21±0.21^{b}
Vitamin C (mg AAE/g)	13.59±0.10^{b}	22.30±0.07^{d}	6.52±0.12^{a}	18.37±0.08^{c}
Total Flavonoids (mg RE/g)	3.10±0.05^{c}	4.94±0.02^{d}	1.16±0.07^{a}	2.84±0.02^{b}
Total Phenolics (mg GAE/g)	3.92±0.01^{c}	5.08±0.08^{d}	1.78±0.01^{a}	3.55±0.06^{b}

Each value is expressed as mean ± standard error (n = 3). Values with different superscript within a row are significantly different at (P < 0.05). Keys: FEA: Acetone leaf extract of Ficus exasperata; FEM: Methanol leaf extract of Ficus exasperata; PAA: Acetone leaf extract of Persea americana; PAM: Methanol leaf extract of Persea Americana; AAE: Ascorbic acid equivalent; RE: Rutin equivalent; GAE: Gallic acid equivalent

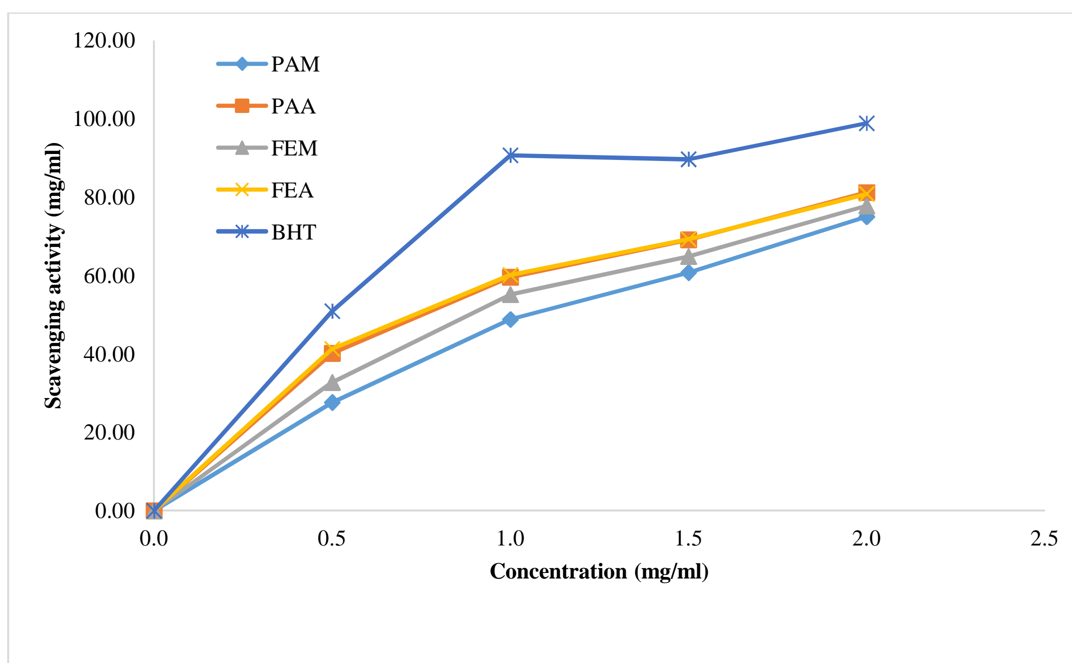

Fig. 2. Scavenging effect of leaves extracts of *Ficus exasperata* and *Persea americana* on DPPH radicals

Each value is mean of triplicate results (n=3). Keys: FEA: Acetone leaf extract of Ficus exasperata; FEM: Methanol leaf extract of Ficus exasperata; PAA: Acetone leaf extract of Persea americana; PAM: Methanol leaf extract of Persea Americana; BHT: Butylated hydroxy toluene (3,5-di-tert-butyl-4-hydroxytoluene); DPPH: (2, 2-diphenyl-1-picrylhydrazyl)

All the extracts were capable of inhibiting malondialdehyde production in a concentration dependent manner. Methanol extract of *P. americana* displayed better inhibitory activity at all tested concentrations. The activity of methanol extract of *P. americana* was close to that of the positive control (BHT) at the similar concentrations (0.5-2 mg/mL).

4. DISCUSSION

The ability of several medicinal plants in reducing oxidative stress has been documented in several reports [32,33]. The present study investigated the ability of leaves of local plants (*Persea americana* and *Ficus exasperata*) collected from Akure in preventing oxidative stress.

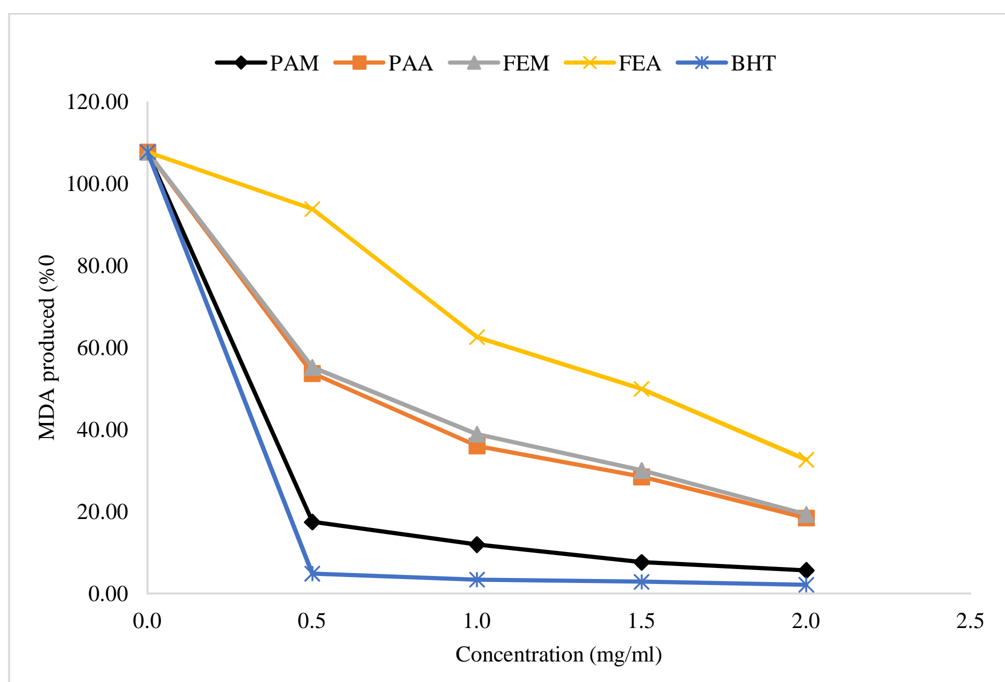

Fig. 3. Inhibition of Fe^{2+} induced lipid peroxidation in rat's liver by leaves extracts of *Ficus exasperata* and *Persea americana*

Each value is mean of triplicate results (n=3). Keys: FEA: Acetone leaf extract of Ficus exasperata; FEM: Methanol leaf extract of Ficus exasperata; PAA: Acetone leaf extract of Persea americana; PAM: Methanol leaf extract of Persea Americana; BHT: Butylated hydroxyl toluene (3,5-di-tert-butyl-4-hydroxytoluene); MDA: Malondialdehyde

The leaves extracts of *Persea americana* and *Ficus exasperata* displayed varying total phenolics, total flavonoids and total vitamin C contents. The variation observed is in accordance to results obtained in similar studies [9,34]. The differences in plants, extraction solvents, extracting capacity of the solvents, sample particle size as well as the presence of interfering substances may account for this variation [35].

Secondary metabolites are produced by plants in order to habituate to conditions such as infection, water stress, and cold stress [5]. These compounds are also capable of protecting the human body from oxidative stress through the removal of free radicals, metal catalyst chelation, oxidase inhibition, and activation of antioxidant enzymes [36].

Result from this study shows that the leaves extracts of *Persea americana* and *Ficus exasperata* contain compounds such as tannin, phenols, polyphenols and vitamin C with reported antioxidant ability [9,34,37]. The potent antioxidant capacity of phenolics for instance, has drawn the attention of several researchers into studying plants secondary metabolites with phenolic functional groups. According to the report of Rice-Evans et al. [38], the structural relationship between different parts of the chemical structures of flavonoids and phenols is chiefly responsible for their antiradical activity. Vitamin C is a potent reducing agent acting as the primary antioxidant in plasma and tissue [39]. It also provides protection to requisite body molecules such as proteins, lipids, carbohydrates and DNA and RNA from damage by free radicals [40]. The presence of phenolics and vitamin C in these plants therefore gives credence to their use in preventing oxidative stress.

The findings from this study reveal the DPPH radical scavenging effect of the leaves extracts of *Persea americana* and *Ficus exasperata*. This ability of the extracts to reduce DPPH corresponds with the findings of Oboh et al. [9] while investigating the antioxidant effect of phenolic extract from leaves and fruits of avocado pear. The reduction of the violet colour of DPPH when mixed with leaves extracts of *Persea americana* and *Ficus exasperata* is an

indication of the presence of substances that can donate hydrogen atom. Substances which are able to perform this reaction possess antioxidant ability and act as radical scavengers [41]. Phenolics, flavonoids and tocopherols have been reported to reduce the DPPH radicals through their hydrogen donating ability [42]. The availability of hydrogen for donation and presence of hydroxyl groups is accredited to the radical scavenging effect of phenolics [43].

Result from the inhibitory effect of the leaves extracts of *Persea americana* and *Ficus exasperata* on lipid peroxidation shows that Fe^{2+} caused a sizeable increase in the amount of MDA content in rat's liver. This observation is in line with the findings of Oboh et al. [9]. Their result while studying the antioxidant ability of phenolic extracts from leaf and fruit of avocado pear (*Persea americana*) implicated iron as a powerful initiator of lipid peroxidation. Iron (II) can catalyse one-electron transfer reactions leading to the generation of highly reactive hydroxyl radical ($OH^{\cdot}$) from hydrogen peroxide leading to increased lipid peroxidation. A sizeable drop in the amount of iron in the body is therefore imperative in reducing oxidative stress as a result of iron overload [44].

All the extracts were capable of lowering the amount of MDA produced from rat's liver, indicating the potentials of these extracts in the protection of the liver against lipid peroxidation. The ability of the extracts to inhibit Fe^{2+} induced lipid peroxidation could be due to the presence of compounds that can form chelates with Fe^{2+}, thereby making Fe^{2+} unavailable for the initiation of lipid peroxidation or through the scavenging of the free radicals generated when Fe^{2+} decomposes hydrogen peroxide.

All the leaves extracts exhibited appreciable Fe^{2+} chelating activity. The ability of an extract to chelate metal ions is determined by how effective the components of the extracts are to compete with ferrozine for ferrous ion. Ferrozine can form complexes with Fe^{2+}, and the red complex formed can be measured at 562 nm. The Fe^{2+} chelating activity of the extracts can be estimated if the extracts contain chelating agents that can compete with ferrozine disrupting the red complex formation [45,46]. The ability of agents in the extracts to chelate and deactivate Fe^{2+}, prevents Fe^{2+} catalysed reaction, and consequently attenuates lipid peroxidation and oxidative stress [47].

The chelating activity of the extracts might be attributed to the presence of phenolic compounds and vitamin C found in the extracts. The compounds have been reported to be excellent chelators of iron [9,48]. In the findings of Adefegha and Oboh [34], spices with high total phenol, flavonoids and vitamin C displayed higher Fe^{2+} chelating activity.

5. CONCLUSIONS

The results obtained from this study reveal that extracts from leaves of *Persea americana* and *Ficus exasperata* possess antioxidant activities *in vitro*. This therefore supports their use in the treatment of radical associated diseases and reduction of oxidative stress.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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