

Original Research Article

Evaluation of the *in vitro* antioxidant activities of leaves extracts of *Persea americana* and *Ficus exasperata* collected from Akure, Nigeria.

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 (Fe²⁺) induced lipid peroxidation were evaluated.

Result: The acetone extract of *F.exasperata* has the highest alkaloids (68.60%), total phenol (5.08mg), total flavonoids (4.94mg) and vitamin C contents (22.30mg). At a concentration of 2mg/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 hydroxy toluene and Ethelenediaminetetraacetic acid) were more effective than the leaves extracts at 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, medicinal plants

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 enormous medicinal potentials of plants, leaves of *Ficus exasperata* and *Persea americana* collected from Akure, were screened for their ability to inhibit Fe^{2+} induced lipid peroxidation in rat's liver *in vitro*.

Materials and Methods

Collection of Leaves Samples

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

68 **Handling and use of animals**

69 The handling and use of animals were in accordance with National Institute of Health (NIH)
 70 Guide for the care and use of laboratory animals. Adult Wister strain albino rats weighing
 71 200–230g were purchased from the Department of Animal production and Health, FUTA.
 72 The rats were housed in a cage with a temperature of $25 \pm 2^{\circ}\text{C}$, on a 12 hour light/12 hour
 73 dark cycle. They were acclimatized under these conditions for two weeks before the
 74 experiment in which the animals were maintained *ad libitum* on commercial food and water.

75 **Preparation of leaves extracts**

76 Powdered leaves (100g) of *Persea americana* and *Ficus exasperata* were weighed separately
 77 into different containers and extracted with 1000ml of 100% acetone and methanol. The
 78 containers were covered with aluminium foil and allowed to stand for 3 days for extraction
 79 with continuous stirring. The solutions were then filtered through Whatman filter paper
 80 (0.45 μm). The filtrates were evaporated to dryness at 50°C in a rotary evaporator (RE-52A;
 81 Union Laboratory, England) with 90rpm under reduced pressure. The obtained concentrated
 82 extracts were stored in dark at 4°C until further analysis.

83 **Determination of Tannins**

84 Tannins determination was done according to the method of Association of Official
 85 Analytical Chemists [AOAC] [18], with some modifications. The sample (0.20g) was mixed
 86 with 20 ml of 50% methanol. This was shaken thoroughly and placed in a water bath at 80°C
 87 for 1 hour to ensure uniform mixing. The mixture was filtered into a 100ml volumetric flask,
 88 followed by the addition of 20ml of distilled water, 2.5ml of Folin-Denis reagent and 10ml of
 89 17% aq. Na_2CO_3 (Sodium carbonate) and was thoroughly mixed. The mixture was made up
 90 to 100ml with distilled water, mixed and allowed to stand for 20 minutes. The absorbance of
 91 the tannic acid standard solutions as well as sample was measured after colour development

at 760 nm using the spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215).

Determination of saponins

Quantitative determination of saponins was done using the method of Obadoni and Ochuko [19]. The powdered sample (20g) was added to 100ml of 20% aqueous ethanol and kept in a shaker for 30 minutes. The samples were heated over a water bath for 4 hours at 55⁰C. The mixture was then filtered and the residue re-extracted with another 200ml of 20% aqueous ethanol. The combined extracts were reduced to approximately 40ml over the water bath at 90⁰C. The concentrate was transferred into a 250ml separatory funnel and extracted twice with 20ml diethyl ether. The ether layer was discarded while the aqueous layer was retained and to which 60ml n-butanol was added. The n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation, the samples were dried in the oven at 40⁰C to a constant weight.

Determination of alkaloids

Alkaloids were quantitatively determined according to the method of Harborne [20]. Two hundred milliliters of 10% acetic acid in ethanol was added to 5g powdered extract, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to one-fourth of its original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was completed and the whole solution was allowed to settle. The collected precipitates were washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

Determination of steroid

Steroid content of the plant sample was determined using the method described by Trease and Evans [21]. A portion of 2ml was taken from a solution of 2.5g of powdered plant material prepared in 50mL of distilled water after vigorous shaking for 1 hour. The extract solution was washed with 3mL of 0.1M NaOH (pH 9) and later mixed with 2mL of chloroform and 3mL of ice cold acetic anhydride followed by the cautious addition of two drops of concentrated-H₂SO₄. The absorbance of both sample and blank were measured using a spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215) at 420 nm.

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 95ml aqueous picric acid + 5ml 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.

Determination of total flavonoid content

The total flavonoid content of the extracts was determined using a colorimeter assay developed by Bao [23]. The extract (0.2ml) was added to 0.3ml of 5% sodium Nitrate (NaNO_3) at zero time. After 5min, 0.6ml of 10% aluminium chloride (AlCl_3) was added, and after 6min, 2ml of 1M NaOH (sodium hydroxide) was added to the mixture followed by the addition of 2.1ml of distilled water. Absorbance was read using a spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215) at 510nm against the reagent blank, and the calibration curve was prepared by using rutin methanolic solutions at concentrations of 12.5 to 100 $\mu\text{g mL}^{-1}$. Total flavonoid was expressed as mg rutin equivalents per gram of dried extract (mg RE g^{-1}).

Total phenolic content Determination

The total phenolic content of the extracts was determined by the method of Singleton *et al.* [24]. About 0.2ml of each of the extracts was mixed with 2.5ml of 10% Folin ciocalteau's reagent and 2ml of 7.5% sodium carbonate (Na_2CO_3). The reaction mixture was incubated at 45°C for 40 minutes, and the absorbance was measured at 700nm in the spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215). A calibration curve was plotted for the standard of gallic acid. Total phenolic content was expressed as mg gallic acid equivalents per gram of dried extract (mg GAE g^{-1}) using the linear equation obtained from standard gallic acid calibration curve.

Determination of Vitamin C content

The method of Benderitter *et al.* [25] was used to determine the vitamin C content of the leaves extracts. Briefly, 75 μL DNPH (2g dinitrophenyl hydrazine, 230mg thiourea and 270mg $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 100mL of 5mol L⁻¹ H_2SO_4) were added to 500 μL reaction mixture (300 μL of an appropriate dilution of the polar extract with 100 μL 13.3% trichloroacetic acid and water). The reaction mixtures were subsequently incubated at 37°C for 3hours. Then

0.5mL of 65% H₂SO₄ (v/v) was added to the medium. Their absorbance was read at 520nm using a spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215). The vitamin C content of the leaves samples was measured using ascorbic acid as the reference compound and expressed as mg/g ascorbic acid equivalent.

Fe²⁺ 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. Freshly prepared 500μM FeSO₄ (150μL) was added to a reaction mixture containing 168μL of 0.1M Tris-HCl (pH 7.4), 218μL saline and the extracts (0 - 25μL). The reaction mixture was incubated for 5 minutes, before the addition of 13μL of 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510nm. EDTA-Na₂ was used as reference standard. The Fe²⁺ chelating ability was then calculated with respect to the reference.

$$\text{Chelating ability (\%)} = \frac{\text{Absorbance of reference} - \text{Absorbance of sample}}{\text{Absorbance of reference}} \times 100$$

DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging activity

DPPH radical scavenging ability of the extracts was determined by the method of Gyamfi *et al.* [28], with slight modification [29]. Briefly, 2ml solution of the extract at different concentrations diluted two-fold in methanol was mixed with 1.0 ml of 0.3mM DPPH in methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 25 minutes. Blank solutions were prepared with each test sample solution (2ml) and 1ml of methanol while the negative control was 1ml of 0.3 mM DPPH solution plus 2ml of methanol. Butylated hydroxytoluene (BHT) was used as positive control. Thereafter, the absorbance of the assay mixture was measured at 518nm against each blank with a UV-visible spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215). Test was carried out in triplicate. DPPH radical inhibition was calculated using the equation:

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

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).

183 **Lipid Peroxidation Assay**

184 **Preparation of tissue homogenate**

185 The rats were decapitated under mild diethyl ether anesthesia and the liver tissue was rapidly
186 dissected and placed on ice and weighed. This tissue was subsequently homogenized in cold
187 saline (1:10 w/v) with about 10-up-and-down strokes at approximately 1200 rev/min in a
188 Teflon-glass homogenizer. The homogenate was centrifuged for 10 min at 3000× g to yield a
189 pellet that was discarded, and a low-speed supernatant (S1) containing mainly water, proteins
190 and lipids (cholesterol, galactolipid, individual phospholipids, gangliosides) was kept for
191 lipid peroxidation assay [30].

193 **Lipid Peroxidation and Thiobarbituric Acid Reactions**

194 The lipid peroxidation assay was carried out by the modified method of Ohkawa [31]. About
195 100µL of S1 fraction (a low-speed supernatant containing mainly water, proteins and lipids
196 (cholesterol, galactolipid, individual phospholipids, gangliosides) was mixed with a reaction
197 mixture containing 30µL of 0.1 M Tris-HCl buffer (pH 7.4), extract (0 - 100µL) and 30µL of
198 the pro-oxidant (250µM freshly prepared FeSO₄). The volume was made up to 300µL by
199 water before incubation at 37⁰C for 1hour. The colour reaction was developed by adding
200 300µL of 8.1% SDS (Sodium duodecyl sulphate) to the reaction mixture containing S1,
201 followed by the addition of 600µL of acetic acid/HCl (pH 3.4) and 600µL of 0.8% TBA
202 (Thiobarbituric acid). This mixture was incubated at 100⁰C for 1 hour. The absorbance of
203 TBARS (Thiobarbituric acid reactive species) produced was measured at 532nm using a
204 spectrophotometer. MDA (Malondialdehyde) produced was expressed as % control using
205 MDA standard curve (0– 0.035 mM).

206 **Statistical analysis**

207 All experiments were carried out in triplicates. Data obtained were analyzed by One Way
208 Analysis of Variance (ANOVA) and means were compared by New Duncan's Multiple
209 Range Test (SPSS version 16). Differences were considered significant at P<0.05.

210 **Results**

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

The methanol extract *Persea americana* displayed the highest amount of saponin (14.22mg/g) while the acetone extract of *Ficus exasperata* gave highest amount of alkaloids (68.60%), vitamin C (22.30mg AAE/g), total flavonoids (4.94mg RE/g) and total phenolics (5.08mg GAE/g). Whereas the highest amount of terpenoids (20.60mg/g), cardiac glycoside (13.49mg/g) and tannin (4.42mg/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 (%)
<i>Ficus exasperata</i>	Methanol	23.19
	Acetone	3.08
<i>Persea americana</i>	Methanol	9.21
	Acetone	5.44

Table 2: The Phytochemical constituents of leaves extracts of *Ficus exasperata* and *Persea americana*

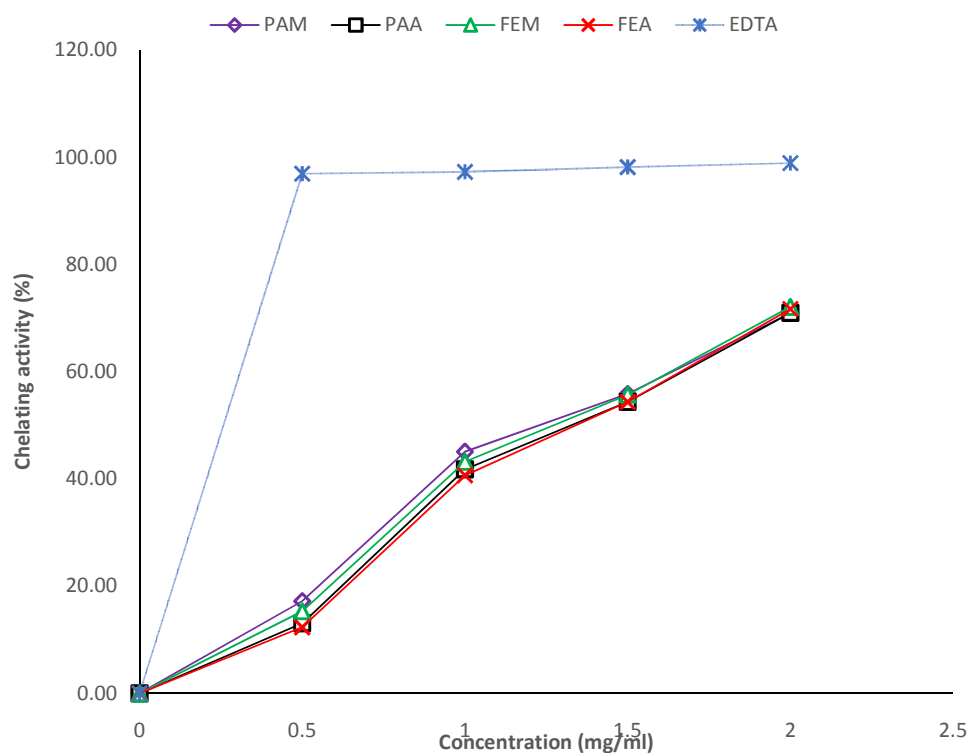
Phytochemical	Extracts			
	FEM	FEA	PAM	PAA
Alkaloids (%)	61.63±0.19 ^b	68.60±3.58 ^c	0.00±0.00 ^a	0.00±0.00 ^a
Terpenoids (mg/g)	10.92±0.19 ^b	15.84±3.58 ^c	11.51±0.00 ^a	20.60±0.10 ^d
Cardiac glycoside (mg/g)	11.09±0.05 ^c	8.31±0.07 ^a	9.94±0.00 ^b	13.49±0.09 ^d
Tannin (mg/g)	4.34±0.06 ^c	2.73±0.05 ^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.

The ferrous chelating effect of the leaves extracts of *F. exasperata* and *P. americana* is displayed in Figure 1. The leaves extracts displayed appreciable iron chelating activity which was higher than 50% at a concentration of 1.5mg/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 2mg/ml.

233 The positive control (EDTA) however displayed higher chelating activities than the extracts
234 at all the tested concentrations (98.87% at 2mg/ml).

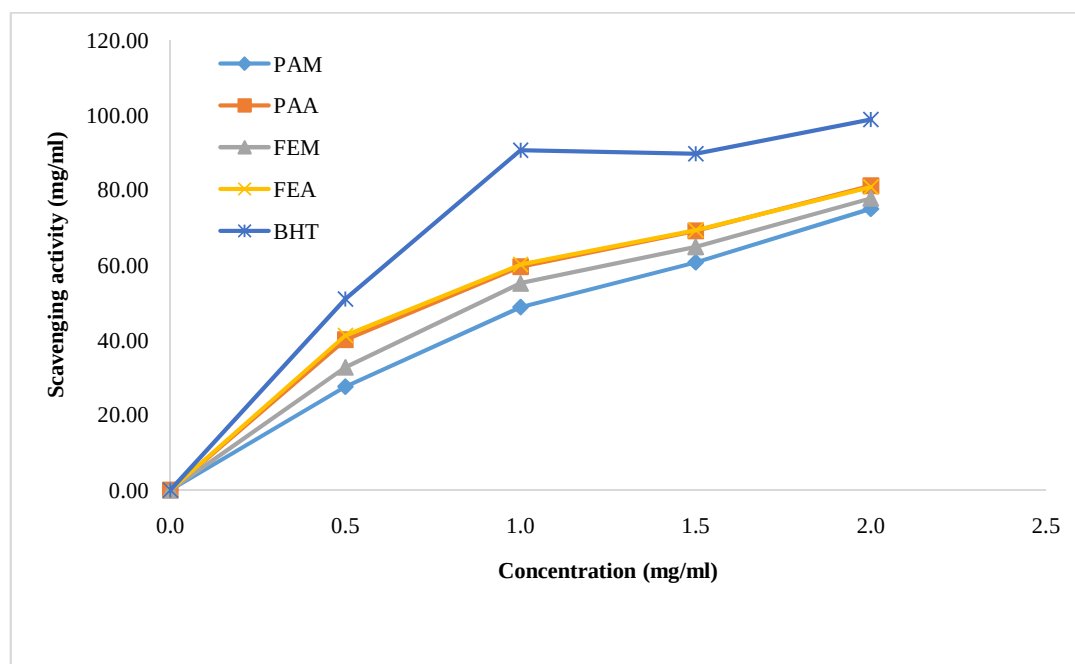
235 The DPPH scavenging activity of the leaves extracts is displayed in Figure 2. The extracts
236 displayed varying and concentration dependent radical scavenging activity. The extracts
237 displayed appreciable radical scavenging activity with the acetone extracts of *F. exasperata*
238 and *P. americana* exhibiting better activities at all test concentrations. At a concentration of
239 2mg/ml, the acetone extract of *P. americana* had the highest activity (81.14%). However, the
240 positive control BHT displayed better and significantly different activity (98.82%) at the
241 same concentration.



242

243 **Figure 1:** Ferrous chelating activity of leaves extracts of *Ficus exasperata* and *Persea*
244 *americana*. Each value is mean of triplicate results (n=3). **Keys:** **FEA:** Acetone leaf extract of
245 *Ficus exasperata*; **FEM:** Methanol leaf extract of *Ficus exasperata*; **PAA:** Acetone leaf
246 extract of *Persea americana*; **PAM:** Methanol leaf extract of *Persea Americana*; **EDTA:**
247 Ethelenediaminetetraacetic acid.

248



249

250 **Figure 2:** Scavenging effect of leaves extracts of *Ficus exasperata* and *Persea americana* on
 251 DPPH radicals. Each value is mean of triplicate results (n=3). **Keys:** **FEA:** Acetone leaf
 252 extract of *Ficus exasperata*; **FEM:** Methanol leaf extract of *Ficus exasperata*; **PAA:** Acetone
 253 leaf extract of *Persea americana*; **PAM:** Methanol leaf extract of *Persea Americana*; **BHT:**
 254 Butylated hydroxy toluene; **DPPH:** (2, 2-diphenyl-1-picrylhydrazyl).

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

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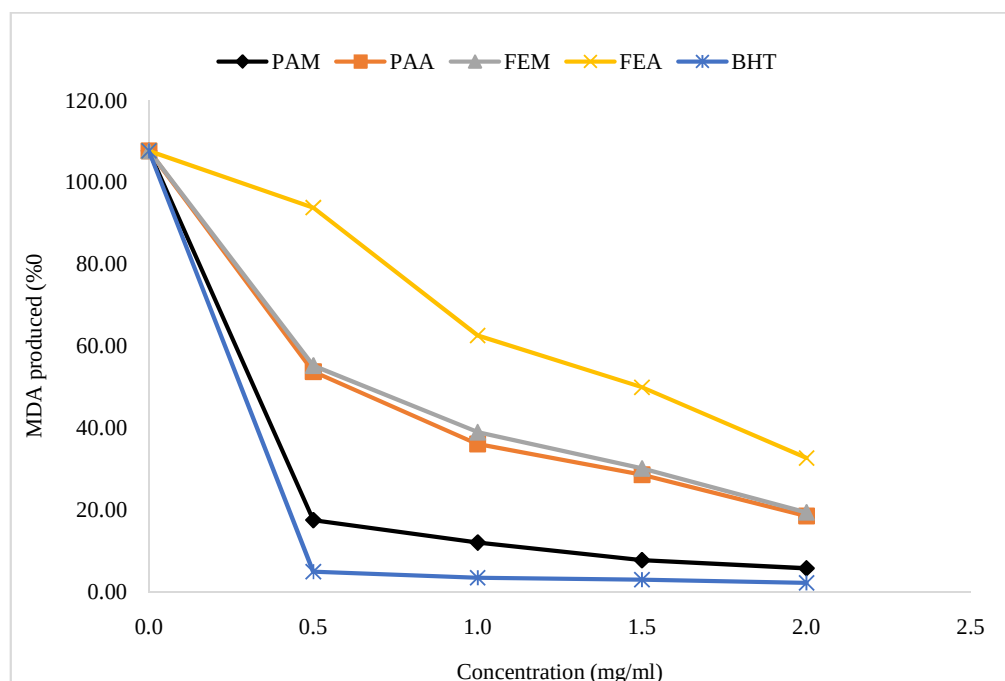


Figure 3: Inhibition of Fe^{2+} induced lipid peroxidation in rat's liver by leaves extracts of *Ficus exasperata* and *Persea americana*. Each value is mean of triplicate results (n=3).
Keys: FEA: Acetone leaf extract of *Ficus exasperata*; FEM: Methanol leaf extract of *Ficus exasperata*; PAA: Acetone leaf extract of *Persea americana*; PAM: Methanol leaf extract of *Persea Americana*; BHT: Butylated hydroxyl toluene; MDA: Malondialdehyde.

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.

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.

Findings from this study reveals 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 ($\text{OH}^\cdot$) 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 562nm. 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.

Conclusion

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

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