Estimation of Total Phenolic, and Total Flavonoid Contents, In -Vitro Antioxidant Activity, and Median Inhibitory Concentration of methanol root extract of *Ximenia Americana,* L. *Olacaceae*.

Abstract

Background: This research was carried out to estimate the total phenolic and flavonoid contents, assayed the in -vitro antioxidant potential, and to estimate the median inhibitory concentration (IC_{50}) of the methanol root extract of *Ximenia americana*, Linn.

Study Design: It was experimental study design.

Methods: Total phenolic and flavonoid contents of the extract was determined by spectrophotometry using Folin-Ciocalteu reagent and Aluminum chloride colorimetric assay, readings were taken at 750 nm, and 415 nm wavelength respectively. The results were expressed in terms of Garlic acid and Rutin standards equivalent. The in-vitro antioxidant activity, and the IC_{50} of the plant material was investigated by its ability to scavenge free radical, using 2,2-diphynyl-1-picrylhydrazyl (DPPH) free radical scavenging method. Similarly, the total percentage yield of the extract was estimated from the crude.

Results: The crude root powder showed a total extract yield of 20.06%. Total phenolic and flavonoid contents were determined to be 3.501 ± 0.774 mg GAE/g, and 10.644 ± 0.20 mg RE/g extract. The extract displayed concentration dependent DPPH free radical scavenging activity, with the highest concentration (200 µg/ml) inhibit 60.93% of DPPH free radical, and the IC_{50} of the extract was determined to be 69.01 µg/ml.

Conclusions: *Ximenia americana* L methanol root extract has a yield of 20.06%, with a total phenolic, content of 3.501± 0.774 mg GAE/g, total flavonoid content of 10.644 ± 0.20 mg RE/g, and IC_{50} of 69.01 µg/ml. also, the extract possesses a potent antioxidant activity by its ability to inhibit DPPH activity.

Key words*: *Antioxidant, DPPH, Total phenolics, Total flavonoids, $IC_{50,}$ Ximenia americana.

1.Introduction

Ximenia americana is a tropical plant, one of the eight species of the *Olacaceae* family. This plant is very useful in traditional medicine and is used differently in different countries predominantly in African to manage varieties of ailments[1] [2] [3]. We have demonstrated the in vivo antioxidant activity of this plant extract using Drosophila melanogaster model [4].

Polyphenolic compounds such as phenolics and flavonoids are a group of phytochemicals that are synthesized by plants to provide immunity for them against adversity[5]. Also, these classes of compounds has been demonstrated to possess antioxidants activity that helps to scavenge or neutralize reactive oxygen and nitrogen (RON) species or free radical[6][7][5][8][9][10]. These secondary metabolites are useful to humans medically to reducing or preventing certain genetic, neurodegeneration, and or chronic diseases such as diabetes, cancer, and immunoregulation[10][11][12][13].

Phenolics, Flavonoids and other polyphenols have been reported to have antibacterial, anti-inflammation, skin protecting effects among other benefits [10].

The total phenolic content of the leaf extract of this plant has been determined[14]. Similarly, work has been done on the anti-nociceptive and anti-inflammatory properties of roots polyphenol fraction in mice[15]. This present work was done in other to estimate the total phenolic and flavonoid contents, and to assay the in-vitro antioxidant activity, hence, established the median inhibitory concentration of the methanol root extract of *X. americana*. Family Olacaceae.

2. Materials and Methods

2.1 *Plant Material Collection and Extraction*

The collection of the plant material, identification/authentication, and the method used for the extraction were previously reported by our team [16].

2.2 *Ethical requirement*

Ethical considerations were not necessary for the collection of the plant material. The plant material was collected in the wild not protected by any law, and this work did not endanger the plant species.

2.3 *Chemical*

The chemicals used in this study include: Rutin, 2,2- diphenyl-1-picrylhydrazyl (DPPH), Sodium Acetate, Sodium Carbonate, Ethanol, Methanol, Ascorbic acid (JHD® CAS No: 50-81-7, Lot No: 20141104), and Aluminum Chloride (D®, CAS No: 7784-13-6, Lot No: 20180604). All the reagents used were of analytical grades.

2.4 *Determination of percentage (%) yield*

The percentage yield of the *Ximenia americana* root extract was determined from the dry weight of the extracted powder(a) and the soaked plant sample powder (b). The formula started below was used:

Percentage yield (%) = dried extract powder weight(a) ÷ weight of soaked plant sample (b) × 100.

2.5 *Total Phenolic Content* (TPC) *Determination*

Total phenolic content (TPC) was determined spectrophotometrically using Folin-Ciocalteu reagent (FCR). The method initially described by[17] and subsequently modified by [8] was

used. First, gallic acid standard curve was plotted from a stock solution with a concentration of 100 mg/ml to make five gallic acid standard solutions of different concentrations (0.02, 0.04, 0.06, 0.08, and 0.10 mg/ml). Next step, 1 ml of each sample as well as diluted plant extract was added to 25 ml volumetric flasks containing 9 ml of distilled H_2O. After that, 1 ml of Folin-Ciocalteu (FC) reagent was added to the flask and mixed thoroughly. Furthermore, the samples were incubated at room temperature for 5 minutes then 10 ml of 7% sodium bicarbonate (Na_2CO_3) solution was added to the mixture in each flask. Subsequently, the flasks were incubated for 90 minutes at ambient room temperature (23˚C). Next, the absorbance of the sample extract, which was orange-yellow in hue, was measured at a wavelength 750 nm using an UV spectrophotometer (Jenway UV-7513). Lastly, the TPC results were expressed as mg/g gallic acid equivalents (GAE/g) of plant root extract using a standard curve. Sample were analyzed in triplicate [18]. The assay was performed in triplicates and the values expressed in mean ± SD.

2.6 *Total Flavonoid Content* (TFC) *Determination*

The total flavonoid content of methanol root extract of *Ximenia americana* was determined using aluminum chloride colorimetric assay procedure previously described by [19][20], and slightly modified by [8]. Briefly, standard solution of quercetin of various concentration (6.25,12.5,18.75,25,50,70 µg/ml) were prepared in 96% ethanol. 50 µl of extracts (1 mg/ml) or standard solution was added to 10 µl of 10% aluminum chloride solution, this was followed by 150 µl of 96% ethanol. Thereafter, 10 µl of 1 M sodium acetate was added to the mixture in the test tubes. 96% ethanol was used as a reagent blank. All reagents were mixed and incubated inside a dark cupboard (to protect each from light) for 30 min at room temperature. The absorbance was measured at 415 nm using a UV- spectrophotometer (Jenway 7513). Rutin was used to make the standard calibration curve.

The total flavonoid content (TFC) was calculated from the calibration curve of Rutin plotted, and the result was expressed in mg Rutin Equivalents (RE) per g of plant extract (mg RE/g). The assay was performed in triplicates and the values expressed in mean ± SD.

2.7 *2,2-diphenol-1-picrylhydrazyl (DPPH) anti-oxidant Assay*

The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was performed using a protocol previously described by [21], and reported by [20][19] [8] with little modification. Briefly, 20 µl of plant extracts (1 mg/ml) or standard solution of ascorbic acid (67 µg/ml) in absolute methanol was added to 180 µl of DPPH reagent in test tubes. Absolute methanol was used for the reagent blank. All reagents were mixed and incubated in a dark cupboard for 30 minutes at room temperature, protected from light. The absorbance was measured at 517 nm with a UV-Spectrophotometer. The experimental setup was done in triplicates. The percentages of the DPPH free radical scavenging activity were calculated as follows:

Percentage Scavenging activity = 100 × (Abs control –Abs sample / Abs control)

The percentages of the DPPH free radical scavenging activity were determined by comparing with free radical scavenging activity of ascorbic acid and expressed as mg vitamin C Equivalent Antioxidant Capacity (VCEAC) per g of dry plant extract. From the result obtained, the median inhibitory concentration required to scavenge 50% ($IC_{50)}$) of the DPPH was estimated.

The results from this research work were analyzed using data analysis from Microsoft excel 16, and GraphPad Prism version 8.0.2 (263) for the IC_{50} analysis.

3 Results and Discussion

3.1 The percentage yield of the methanol extract of the plant was found to be 20.06%. This means that in every 100 gm of crude dried plant powder, after extraction, 20.06% of the dried extract powder was obtained.

Fig. 1 Cleaned root of *Ximenia americana*.

3.2 *Total Phenolic Content*

The total phenolic content of the plant extract was assayed in order to figure out the amount of phenolics content in it, this is a correlation to the antioxidant capacity of the plant extract.

Fig. 2 showed the equation of the garlic acid calibration curve. From the regression equation of the calibration curve (Y= 0.17x + 0.0944, and a correlation coefficient, R^2 = 0.9746), the total phenolic content was estimated to be 3.501 ± 0.774 mg GAE/g (Table 1). With the high correlation coefficient (R^2) 0.9746, it can be deduced that the extract possesses a good antioxidant potential.

3.3 *Total Flavonoid Content*

Flavonoid is the largest class of polyphenolic compounds that is well distributed in plants[9][22]. This class of polyphenolics is made up of about six sub-classes including; anthocyanins, flavanols, flavanones, flavones, flavonols, and isoflavones [5][23][24].

Fig. 3 showed the equation of the Rutin calibration curve, and from the regression equation of the calibration curve (Y= 0.0177x + 0.0536, and a correlation coefficient, R^2 = 0.998), The total flavonoids contents in the methanol plant extract was calculated to be 10.644 ± 0.20 mg RE/g (Table 1).

3.3 *In vitro antioxidant (DPPH) Assay*

The antioxidant activity of the methanol root extract of *X. americana* was determined via DPPH free radical scavenging assay. The ability of the tested extract to scavenged DPPH free radical is shown in Fig. 4. Our result demonstrated a dose-dependent increase in antioxidant activity of the test material. The highest concentration of the extract (200 µg/ml) scavenged DPPH radical by 60.93% as compared to the standard ascorbic acid (67 µg/ml) that showed 100% DPPH scavenging. Similarly, the median inhibitory concentration (IC_{50}), of the plant extract was 69.01 µg/ml, with a correlation coefficient (R^2) of 0.9551 (Fig. 5). These results showed that the plant material has a potent antioxidant activity, and a good safety margin which correlated well with the IC_{50}.

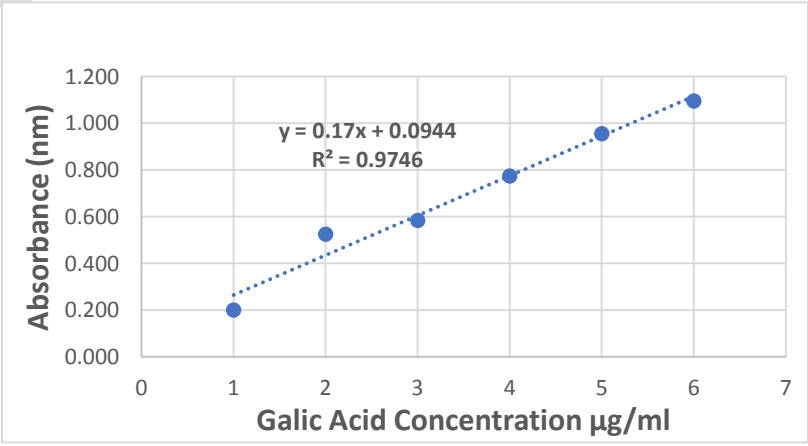

Fig. 2 Garlic acid calibration curve.

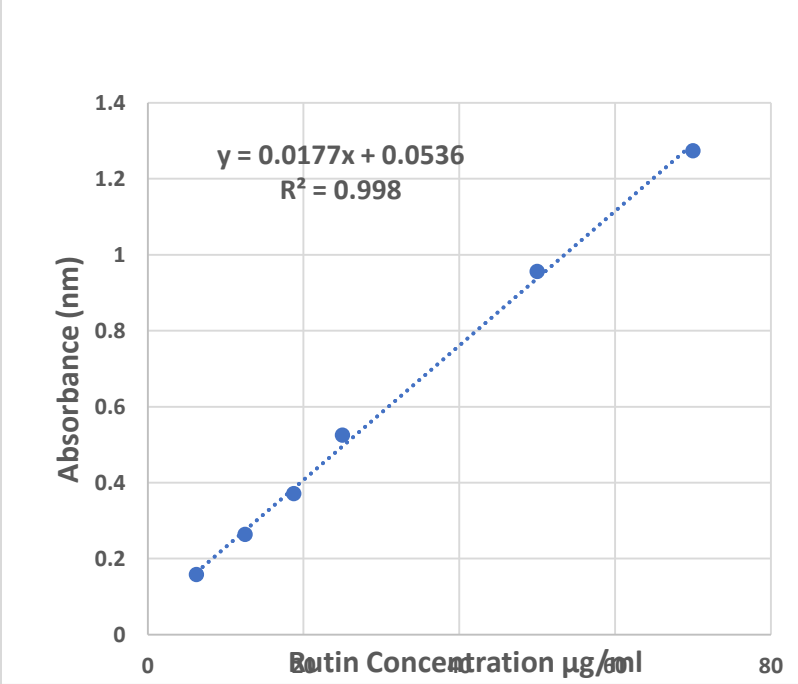

Fig. 3 Rutin calibration curve

Table 1. Total phenolic and flavonoid contents of the *X. americana* extract

Methanol Extract	Total Content
Phenolic (mg GAE /g)	3.501 ± 0.774
Flavonoid (mg RE/g)	10.644 ± 0.20

GAE = Garlic Acid Equivalent

RE = Rutin Equivalent

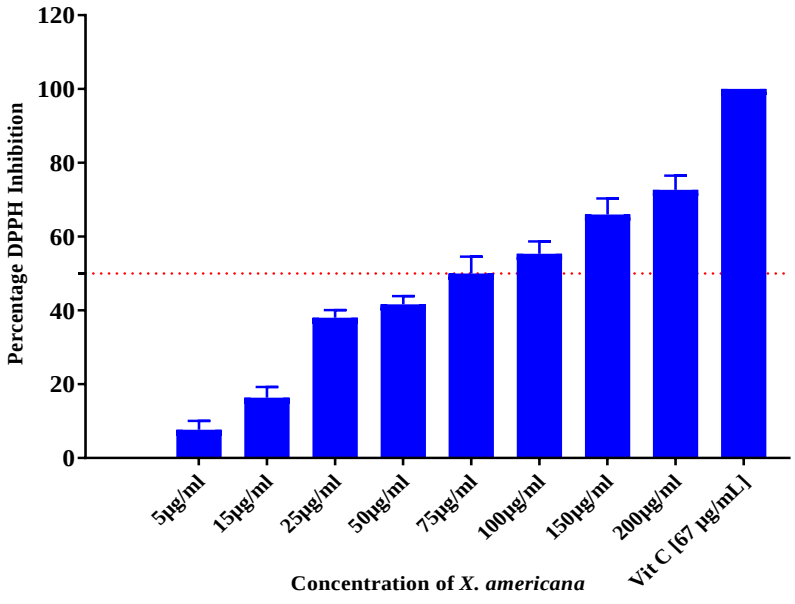

Fig. 4 Percentage DPPH activity of *X. americana*.

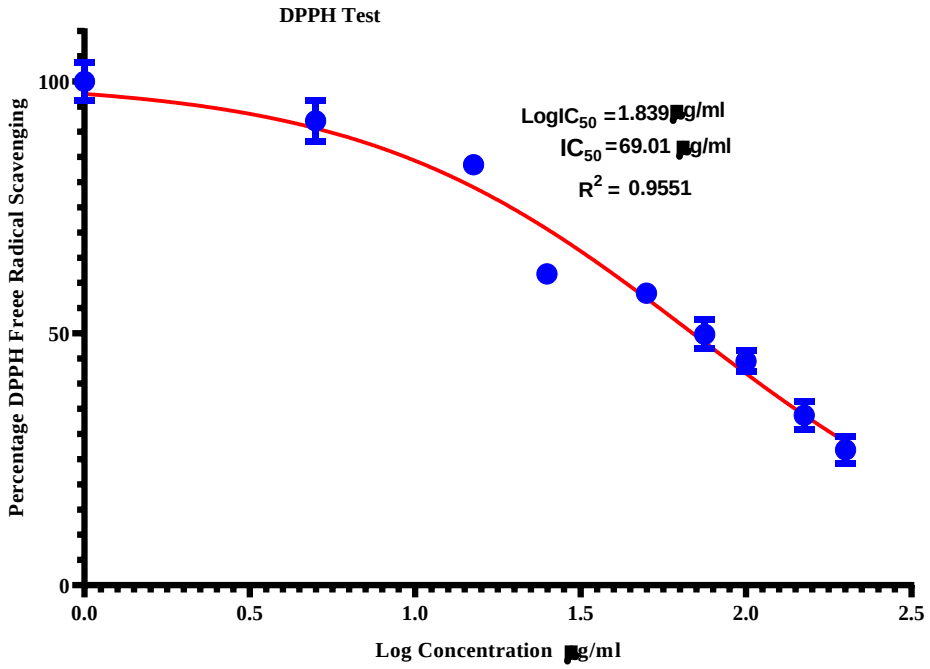

Fig. 5 DPPH median inhibitory concentration (IC_{50}) of *X. americana* root extract

4.0 Conclusion

This study has unraveled the presence and quantities of total phenolics and flavonoids that are content in the methanol root extract of *Ximenia americana*, elucidate by in-vitro its antioxidant potentials, and estimated the concentration of the extract that can inhibits 50% DPPH activity. The antioxidant potential of the plant may be due to the rich phenolics and flavonoids content, hence, its ability to scavenged DPPH free radicals. The presence of this phytochemical in this plant extract could be a reason why it is used in folk medicine for management of many disease conditions, especially those that are associated with oxidative stress. Further work needs to be done to isolate and characterize the bioactive compounds that are responsible for the observed antioxidant activity of this plant part.

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