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**Original Article**

# Evaluation of the antioxidant activity of total aqueous extract of *Bridelia ferruginea* benth. (Euphorbiaceae)

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## ABSTRACT

*Bridelia ferruginea* benth. (Euphorbiaceae) is a medicinal plant used in African traditional medicine to cure fevers. Its extract used in this study was powdered form obtained from the maceration of the dried plant bark in water, followed by evaporation to dryness. In this study, the antioxidative and radical scavenging activities of the total aqueous extract were studied throughout six standard biological tests: DPPH reduction, ferric thiocyanate (FTC) lipidic peroxidation inhibition and thiobarbituric acid reacting substances (TBARS), reducing power, Chelating power and the FRAP method. Vitamin C was used as a reference. Our results showed that *B. ferruginea* has a very high content of phenolic compounds (169.00 ± 0.00 mg GAE / g of extract, 11.72 ± 1.54 mg QE / g of extract and 78.33 ± 11.45 mg QE / g of extract phenols, flavonoids and total flavonols respectively) and a high antioxidant activity. Indeed concentrations inducing 50% reduction of DPPH and chelation of Fe II ($IC_{50}$) were 6.38 ± 0.02 µg / mL and 13.20 ± 0.53 µg / mL respectively with almost 72 % inhibition of lipid peroxidation. This activity is substantially similar to that of the reference molecule, Vitamin C (1.95 ± 0.63 µg / mL, 05.38 ± 0.08 µg / mL and more than 75% inhibition of lipid peroxidation). The result shown by the total aqueous extract of this medicinal plant therefore gives us hope in the search for new and efficient antioxidants molecules.

**Keywords**: *Bridelia ferruginea*, oxidative stress, antioxidant activity, chelating power, anti-radical activity, reducing power.

## INTRODUCTION

Reactive oxygen species are prominent in cellular metabolism. According to **[1],** superoxide anion ($O_2$°-) produced in the mitochondrial respiratory chain or during the phagocytosis is necessary for the performance of the immune system. In physiological conditions, the production of free radicals is controlled by an antioxidant defense system to maintain optimal functioning of the organism. Meanwhile, overproduction of these radical species leads to oxidative stress.
There are numerous conditions under which oxidative stress can become a real heath problem.
Defined as the imbalance in the balance of antioxidant/free radicals towards the latter, oxidative stress causes extensive damage to biological molecules such as DNA, lipids and proteins. Thus it is the cause of several diseases including cancer, cataracts, amyotrophic lateral sclerosis, accelerated aging, the acute respiratory distress syndrome and pulmonary oedema **[2]**. Moreover, in a recent study, a link between oxidative stress and male infertility problems was established **[3]**.

In view of the significant damage caused by oxidative stress, recently researchs have been undertaken for antioxidant compounds to fight it. Currently a variety of synthetic antioxidant supplements are marketed to balance oxidants/antioxidants, but unfortunately these substances have been suspected of toxic effects. This is the case of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and gallic acid esters, all of which are suspected of having negative health effects **[4]** and consequently, strict restrictions on the use of these substances have been implemented in several countries such as Japan, Australia, Rumania, Sweden **[5,6]** and Pakistan. In Pakistan, for example, their use is prohibited for their endocrine disruptive effects **[7]**.

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However, the use of these synthetic antioxidant molecules is not without risk, due to the potential toxicological risks **[8]**. In addition, these synthetic antioxidants have shown low solubility and only moderate antioxidant activity **[9]**. Thus, the need to replace them with natural antioxidants becomes necessary.

Furthermore, the phenolic compounds found in medicinal plants, are known for their good antioxidant activity and could serve as a potential source of new natural antioxidant substances. This fact motivated our research team to evaluate the antioxidant activity of *Bridelia ferruginea* Benth. (Euphorbiaceae), a medicinal plant used in the Ivorian traditional medicines for its various pharmacological properties **[10-11]**. Recently, researchers have focused on natural antioxidants, including those from plants. According to **[12]**, the antioxidant activities of plants are mainly due to the presence of secondary metabolites of the polyphenol class. These antioxidants can play an important role in the prevention and therapy of various diseases. In order to provide a solution to this problem, we suggest to contribute to the search for new antioxidants. The aim of our study was to determine the polyphenol contents (phenols, total flavonoids and flavonols) and to assess the antioxidant activity *in vitro* of total aqueous extract of this plant.

## MATERIALS AND METHODS

***Plant Material:*** The plant material used consisted of some powder of stem barks of *Bridelia ferruginea* Benth. (Euphorbiaceae).

***Technical materials:*** The technical materials used include a mechanical grinder IKAMAG type, a Rotavapor Laborata 4000 & WB Heidolph, a UV-Vis spectrophotometer Jr bioMérieux of SECOMAM, a DRIG-BIG oven by P SELECTA and brand Denver weighing scale Accuracy Instrument = 0.001 g.

***Reagents:*** The reagents used were mainly the Folin-Ciocalteu reagent, sodium carbonate, methanol, aluminum chloride, potassium acetate, sodium acetate, 2,2-diphenylpicrylhydrazyl (DPPH), the phosphate buffer, ethanol, hydrochloric acid, ferrous chloride, ammonium thiocyanate, ethylendiamine tetra-acetic acid (EDTA), thiobarbituric acid (TBA), trichloroacetic acid (TCA), the ferosine, linoleic acid. Apart from DPPH which was supplied by Sigma (St. Louis, United States of America), all other chemicals were obtained from E. Merck PROLABO and Darmstadt.

***Preparation of the plant extract:*** The basic method was consisted of extracting a substance contained in a solid body using a solvent. Thus, after harvesting, cleaning and drying in the dark, the *Bridelia ferruginea* stem bark were ground into fine powder using an electronic blender IKAMAG to obtain plant powder. To 100 g of this powder was added one liter of distilled water. After homogenization, the mixture was stirred using a magnetic stirrer during 24 hours for complete extraction. Thereafter, three successive filtrations were performed twice through cotton wool and once with a 3mm Whatman paper. The filtrate was, concentrated by evaporation under vacuum at 30 ° C using a rotary evaporator (Rotavapor) Büchi. The resulting paste was incubated in an incubator at 50 ° C during 24 hours. This method was developed by **[13]**.We thus obtained total aqueous extract which was used for the preparation of solutions of different concentrations needed to perform various *in vitro* antioxidant activity test.

***Determination of total phenols:*** The total phenols content in the total aqueous extract of the plant was determined by the Folin-Ciocalteu method **[14]**. To 0.5 mL of the plant extract of concentration 0.1 g / mL were respectively added 5 mL of Folin-Ciocalteu reagent diluted 1:10 in distilled water and 4 mL of sodium carbonate (1 M). The whole mixture is incubated at room temperature during 15 minutes. The optical densities (OD) were then read with a spectrophotometer at 765 nm against a blank. Gallic acid, which is our standard, was prepared in the same conditions as above with a solvent mixture of methanol / water (50: 50, V/V) at concentrations ranging from 0 to 200 mg / mL. The total phenolic content of the plant extract was expressed in milligram of gallic acid equivalents per gram of extract (mg GAE / g of extract).

***Determination of total flavonoids:*** The technique used for the determination of the total flavonoid content of the extract of *B. ferruginea* is the colorimetric method in aluminum chloride as described by **[15]**. Thus to 0.5 mL of (0.1 mg / mL) of the plant extract was collected, to which are added 1.5 mL of methanol; 0.1 mL of 10% aluminum chloride, 0.1 mL of potassium acetate (1M) and 2.8 mL of distilled water successively. After incubation at room temperature during 30 minutes, the optical densities were measured with a spectrophotometer at 415 nm. Methanolic solution of quercetin in concentrations ranging from 0 to 100 µg / mL was used as a standard. The flavonoid content of the extract is expressed as milligram of quercetin equivalent per gram of extract (QE mg / g of extract).

***Determination of total flavonols:*** The total flavonol content was determined using the method of **[16]**. To 2 mL of extract (0.1 mg / mL) are added 2 mL of an ethanolic solution of 2% aluminum chloride and 3 mL sodium acetate (50 g / l). After 2 hours 30 minutes incubation at 30 ° C, the absorbance was measured with a spectrophotometer at 440 nm. Quercetin prepared at concentrations ranging from 0 to 100 µg / mL was used as standard. The total flavonol content of the plant extract is expressed in milligram of quercetin equivalent per gram of extract (QE mg / g of extract).

***Measurement of anti-radical power:*** The measurement of the anti-radical activity of the plant extract was performed by testing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method of **[17].** The idea was to evaluate the ability of the extract to fix free radicals the measurement of the decreasing into the violet color due to the reduction of the radicals produced by the DPPH. Thus, from a stock solution of the plant extract of 0.1 mg/mL, a concentration range was prepared by successive double dilution. Then at each concentration of extract, the same volume of methanolic solution of DPPH was added. After 30 minutes incubation at room temperature (37 ° C) and protected from light, the absorbance was read with a spectrophotometer at 517 nm against a blank sample (0 mg / mL of extract). Vitamin C (0 to 0.1 mg / mL) prepared in the same conditions is used as a reference solution.
The percentage inhibition of DPPH radical is calculated by the following formula:

**Inhibition = [(white ABS - ABS sample) / white ABS] x 100**

where white ABS is the absorbance of the blank sample and ABS sample represents the absorbance of the plant extract and vitamin C.
From a graphical curve representing the percentage of inhibition of DPPH radicals against concentrations of plant extract and vitamin C, concentrations of plant extract and vitamin C reducing 50% of DPPH radical ($IC_{50}$) are determined and compared.

***Measurement of the reducing power:*** The method described by **[18]** was used to measure the reducing power of the plant. It measures the ability of the extract to reduce ferric ion ($Fe^{3+}$) to ferrous ion ($Fe^{2+}$).Thus 1 mL of plant extract and vitamin C at different concentrations were separately mixed with 1 mL of phosphate buffer (0.2 mM; pH 6.6) and 1 mL of 1% potassium ferricyanide. The mixture was incubated in a water bath at 50 ° C during 30 minutes. After addition of 1 mL of 10% trichloroacetic acid, the reaction mixture was centrifuged at 3000 rev / min during 10 minutes. To the supernatant was added 0.1 mL of 0.1% ferric chloride and 2 mL of distilled water. After 10 minutes incubation at room temperature, the absorbance was measured with spectrophotometer at 700 nm against a blank containing no extract.
The increasing in absorbance of the sample indicates an increasing in reducing power according to the concentrations of the extract and vitamin C. The concentration inducing an absorbance of 0.5nm ($EC_{50}$) of the sample was determined and compared to that of vitamin C.

***Measurement of the chelating power:*** The colorimetric method of **[19]** based on the determination of the complex formed by the ferrous ion ($Fe^{2+}$) and the ferrosine was used to measure the chelating power of the plant extract. Thus, 3.7 mL of methanol, 0.1 mL of iron II chloride (2 mM) and 0.2 mL of ferrosine (5 mM) were added successively to 1 mL of plant extract at different concentrations (0 to 800 µg / mL) to initiate the reaction. After vigorous stirring and then incubated at room temperature during 10 minutes, the absorbance was read with a spectrophotometer at 562 nm against a blank. Vitamin C at different concentrations was used as a reference solution. The chelating power of the samples was determined using the following formula:

**Power chelator (%) = [(white ABS - ABS sample) / white ABS] x 100**
The concentration of plant extract capable of binding 50% of metals ($IC_{50}$) was determined and compared to that of vitamin C.

***Measurement of the lipid peroxidation inhibition:***
Lipid peroxidation inhibition measurement was assessed by two methods: the ferric thiocyanate test (FTC) which leads to measure the level of peroxides formed by determination of the ferrous ion complex and ammonium thiocyanate, and the thiobarbituric acid test (TBA) which consists of measuring the complex formed between thiobarbituric acid and one of the breakdown products of lipid peroxidation that is malondialdehyde (MDA) in this experiment **([20]).**

***Ferric thiocyanate method (FTC):*** In bottles protected by foil paper were dissolved separately 4 mg of plant extract in 4 mL of 99.5% ethanol to whichwere successively added 4.1 mL of 2.5% linoleic acid in (99.5%) ethanol, 8.0 mL of phosphate buffer (20 mM,pH 7, 0) and 3.9 mL of distilled water for getting a final volume of 20 mL. Vitamin C (reference compound), prepared under the same conditions was used as positive control. The bottles containing mixtures were incubated in a

water bath at 45 ° C. During incubation, at every 24 hrs, 0.1 mL of the mixture was collected in test tubes to whichwere successively added 9.7 mL of (75%) ethanol, 0.1 mL of ammonium thiocyanate (30% in distilled water) and 0.1 mL of iron II chloride ($FeCl_2$) of 20 mM in 3.5% HCl. After 3 minutes incubation at room temperature, the absorbance of the resultant red color was read in a spectrophotometer at 500 nm. Assays were performed for 7 days until the absorbance of the negative control (4 mL of ethanol) reaches its maximum value.

***Thiobarbituric acid method (TBARS):*** This test was used to assess the inhibitory potential of the plant extract by the assay of the complex formed between the thiobarbituric acid and malondialdehyde that is one of the decomposition products of peroxides during lipid peroxidation. The measurement was done in the last day of the FTC test (7th day). Thus, to 2 mL of the mixture were added to 1 mL of an aqueous solution of trichloroacetic acid (20%) and 2 mL of an aqueous solution of (0.67%) thiobarbituric acid. The mixture was then incubated in a hot water bath (100 ° C). After 10 min incubation followed by centrifugation at 3000 rev / min for 10 min, the supernatant was recovered for reading on a spectrophotometer at 532 nm against a blank containing ethanol in the place of the mixture.
The inhibition percentage of lipid peroxidation was determined using the following formula:

**Inhibition (%) = [1 - (ABS sample / white ABS) x 100]**

***Measuring the total antioxidant capacity:*** The ferric reducing activity of plant was determined by the method of **[21]**. It is based on measuring the ability of the extract to reduce the ferric ion (Fe3 +) to ferrous ion (Fe2 +) by the test of FRAP (ferric Reducing activity power). This test measures the change in absorbance at 593 nm of a blue complex formed by the iron II and tripyridyltriazine (TPTZ) by the action of antioxidants electron donors. The FRAP solution consists of 300 mM acetate buffer pH6,6 (3.1 g of $CH_3COONa$ and 16 mL of $CH_3COOH$), 10mM of TPTZ (2, 4,6-tripyridyl -5-triazine) in 40 mM of HCl and 20 mM $FeCl_3$ -6 H2O, in the following amounts of 25 ml acetate buffer, 2.5 mL and 2.5 mL of TPTZ and $FeCl_3$ 6 H2O. To 0.2 Ml volume of plant extract were added 2.8 mL of the FRAP solution (freshly prepared and previously incubated at 37 ° C before use). After 30 min incubation, the optical densities were read at 593 nm against a blank consisting of 2.8 mL of FRAP and 0.2 mL of distilled water. The iron II solution (0.2 to 1 mM) prepared from ferrous sulfate in distilled water (FeSO4, 7 H2O) was used as standard. Data were expressed as iron II µmol per g of sample and compared with the vitamin C.

***Statistical analyses:*** Graphical representations of the data were drawn using the Graph Pad Prism 5.0 software (Microsoft, USA). The mean value is recorded with the standard error of the mean (Mean ± SEM). Statistical analyses of results were performed using analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The significance of analyses is set at $P < 0.05$.

## RESULTS AND DISCUSSION

***Polyphenol content of the plant:*** The polyphenol content of *Bridelia ferruginea* were determined by projecting the optical densities obtained on the calibration curves of gallic acid and quercetin (Figure 1, 2 and 3).

***Anti -radical and iron chelating activities of plant:*** The anti-radical capacity and chelating of total aqueous extract of *Bridelia ferruginea* are shown in Figures 4 and 5. The concentrations of the plant extract causing 50% reduction of DPPH radicals and chelating ($IC_{50}$) are listed in Table II.

***Reducing power:*** Reducing power measurement is used to evaluate the capacity of the plant extract and vitamin C to reduce ferric ion to ferrous ion by the increase in green chromophore coloration. The plots of absorbance against concentrations of the plant extract and vitamin C graph indicated a very high reducing power of *Bridelia ferruginea* and similar to that of vitamin C with the effective concentrations resulting in absorbance of 0.5 ($EC_{50}$ ) 9.23 µg / mL for the plant extract and 3.12 µg / mL for Vitamin C.

***Total antioxidant power:*** The results showed that the *Bridelia ferruginea's* aqueous extract has a total antioxidant power of 1040 µmol Fe II while Vitamin C exhibits a total antioxidant power of 1640 µmol Fe II (Figure 7).

***Lipid peroxidation inhibition power:*** The inhibition of peroxidation (absorbance) against number of days (Figure 8) showed that the curves of the plant extract have a similar appearance to that of Vitamin C and well below that of the negative control. This observation was confirmed by the TBARS method which showed no difference between the percentage of inhibition of lipid peroxidation of *B. ferruginea* (71.91 ± 0.78%) and Vitamin C (75.17 ± 2.30%).

This study highlighted the antioxydant activity of *Bridelia ferruginea* starting from six biological

tests which are the reduction of radicals DPPH, the ions ($Fe^{3+}$), the chelating activity of the metal ions such as the ferro-ion ($Fe^{2+}$), the inhibition of the lipidic peroxidation (FTC and TBARS) and the content of total phenols of the plant.

Indeed, this study showed that *Bridellia ferruginea*'s aqueous extract has a high antioxidant activity by the high reducing activity on DPPH radicals and Ferric ions ($Fe^{3+}$). This potential is due to its high polyphenol content as revealed by its phenolic compound essay and phytochemical screening study by **[6]**.

Indeed, there is a linear correlation between the polyphenols contents of plants and their antioxidant activity through the reducing activity. According to **[22]**, the functional groups present in the polyphenol structures can easily give an electron or a proton to neutralize free radicals. Several other authors have shown that inhibition of the production of reactive oxygen species (ROS) by polyphenols may start by formation of complex inhibitors-enzymes and / or by direct free radical scavenging **[23].**

The antioxidant activity can be also evaluated by the measurement of the chelating activity of metal ions such as ferrous iron ($Fe^{2+}$), indicates that the total aqueous extract of the plant species has a good chelating power, so a good antioxidant power. These results could be explained by the presence of a large proportion of phenolic compounds in the plant extract.

Our results are in accordance with those of **[24]** who showed that the green tea polyphenols confers its good antioxidant activity to it. For this author, these polyphenols are able to inhibit the oxidative stress by chelating the metal ions to inert forms.

The measurement of the inhibition of lipidic peroxidation for the evaluation of the antioxidant power of the vegetal extract has shown that total aqueous extract of *Bridellia ferruginea* has a high inhibition of lipid peroxidation substantially similar to that of vitamin C, which is a reference antioxidant molecule. This inhibitory activity is due to its high polyphenol content especially the presence of total flavonoids because there is a linear correlation between the lipid peroxidation inhibitory power of medicinal plants and their total flavonoids content. Thus it is possible to draw the conclusion from the presence of the good antioxidant activity. Indeed, according to **[24]**, polyphenols in general and flavonoids in particular, are thermodynamically capable to reduce the peroxide radicals by electron transfer due to their low redox potential.

## CONCLUSION

This study on the assessment of antioxidant activity *in vitro* of *Bridellia ferruginea* showed the presence at high levels of phenolic compounds including phenols, total flavonoids and flavonols with very high antioxidant activities and substantially similar to those of vitamin C which is a reference molecule. This medicinal plant, whose total aqueous extract is studied here, could be a promising source of new natural antioxidants molecules needed to fight against metabolic diseases related to oxidative stress. However, antioxidants *in vivo* tests are needed to confirm the *in vitro* antioxidant activity here observed.

**Table I: LEVELS OF PHENOLIC COMPOUNDS OF THE PLANT EXTRACT REPRESENTING AVERAGE OF THREE EXPERIMENTS WITH STANDARD DEVIATIONS.**

| | Phenolic compound | | |
|---|---|---|---|
| | **Total Phenols (µg GAE/g of'extract)** | **Total Flavonoïdes (µg QE/g of extract)** | **Total Flavonols (µg QE/g of extract)** |
| ***Bridelia ferruginea*** | 169,00 ± 0,00 | 11,72 ± 1,54 | 78,33 ± 11,45 |

**Table II: CONCENTRATIONS INDUCING 50% REDUCTION IN DPPH AND CHELATOR, IC50 OF THE PLANT EXTRACT**.

| | $IC_{50}$ (µg/mL) | |
|---|---|---|
| | **Anti-radical activity** | **Chelating power** |
| ***Bridelia ferruginea*** | 6,38 ± 0,02 * | 14,80 ± 0,53** |
| **Vitamine C** | 1,95 ± 0,63 * | 03,75 ± 0,08* |

*: No significant difference; **: significant difference ($\alpha = {}^{0}/_{00}$)

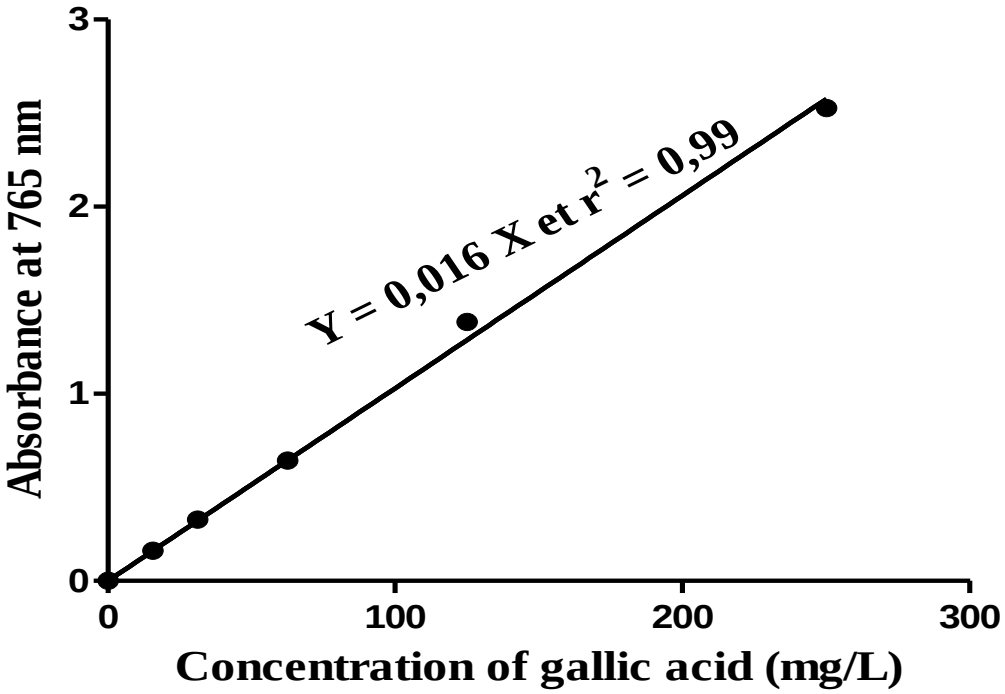


**Figure 1: Callibration curve of gallic acid**

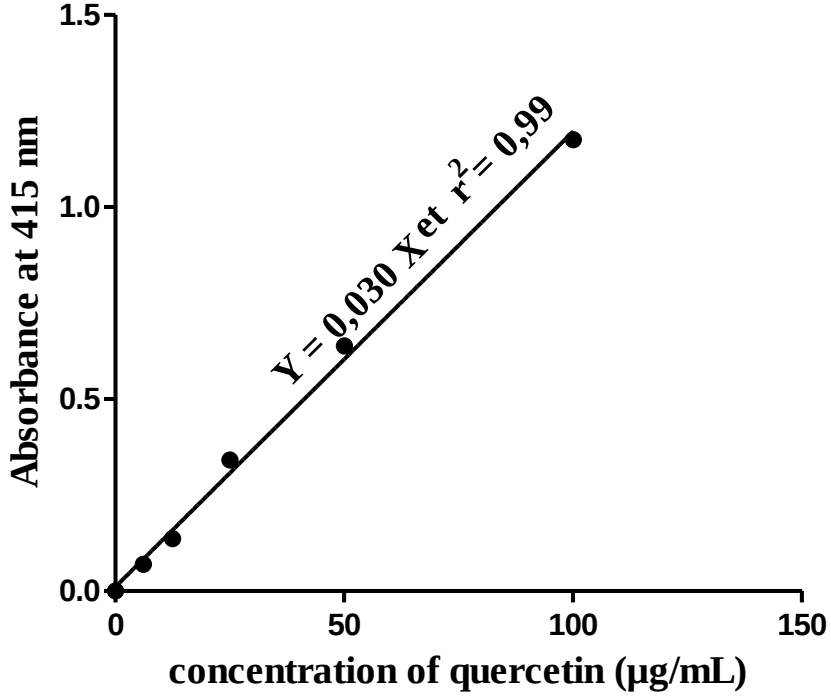


**Figure 2: Callibration curve of quercetin**

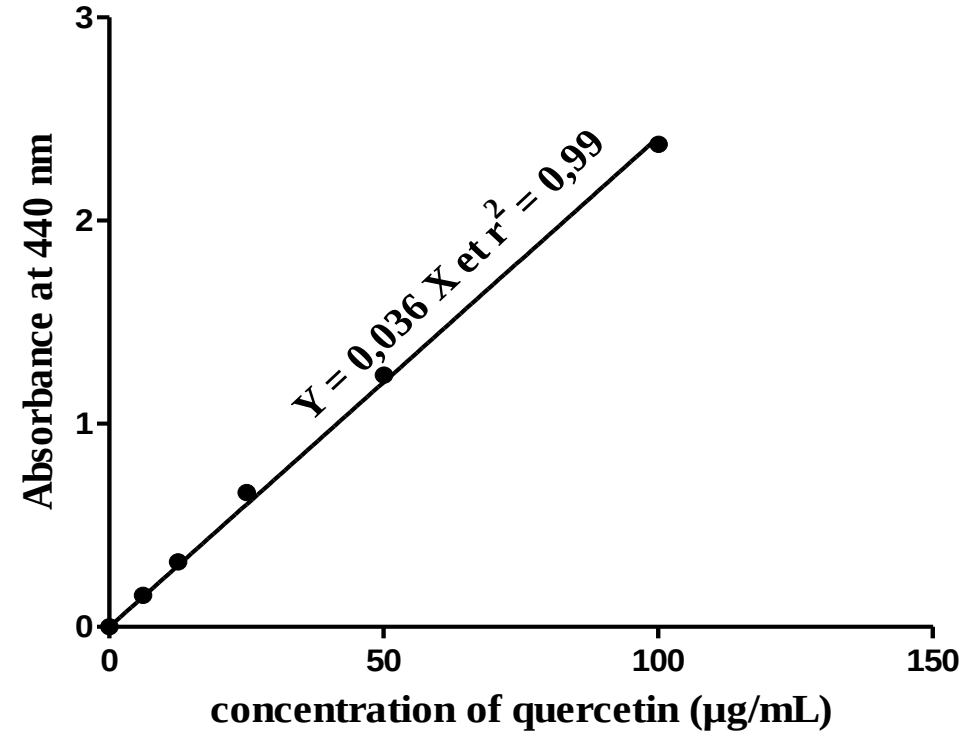


**Figure 3: Callibration curve of quercetin**

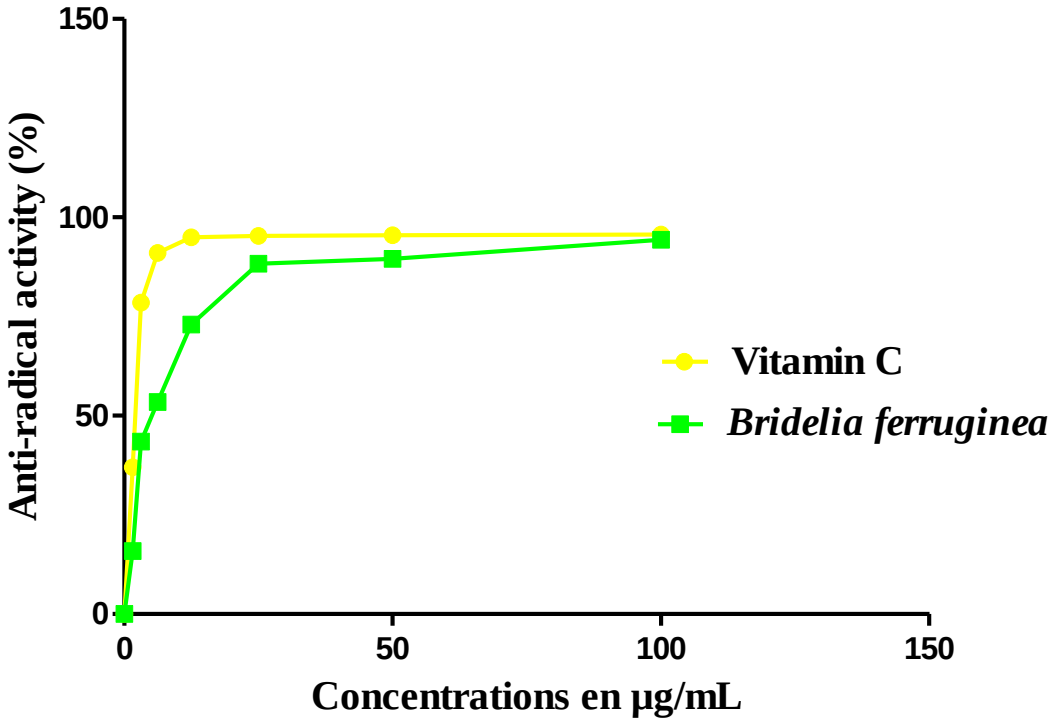


**Figure 4:Anti-radical power of *Bridelia ferruginea* in comparison of those of vitamin C**

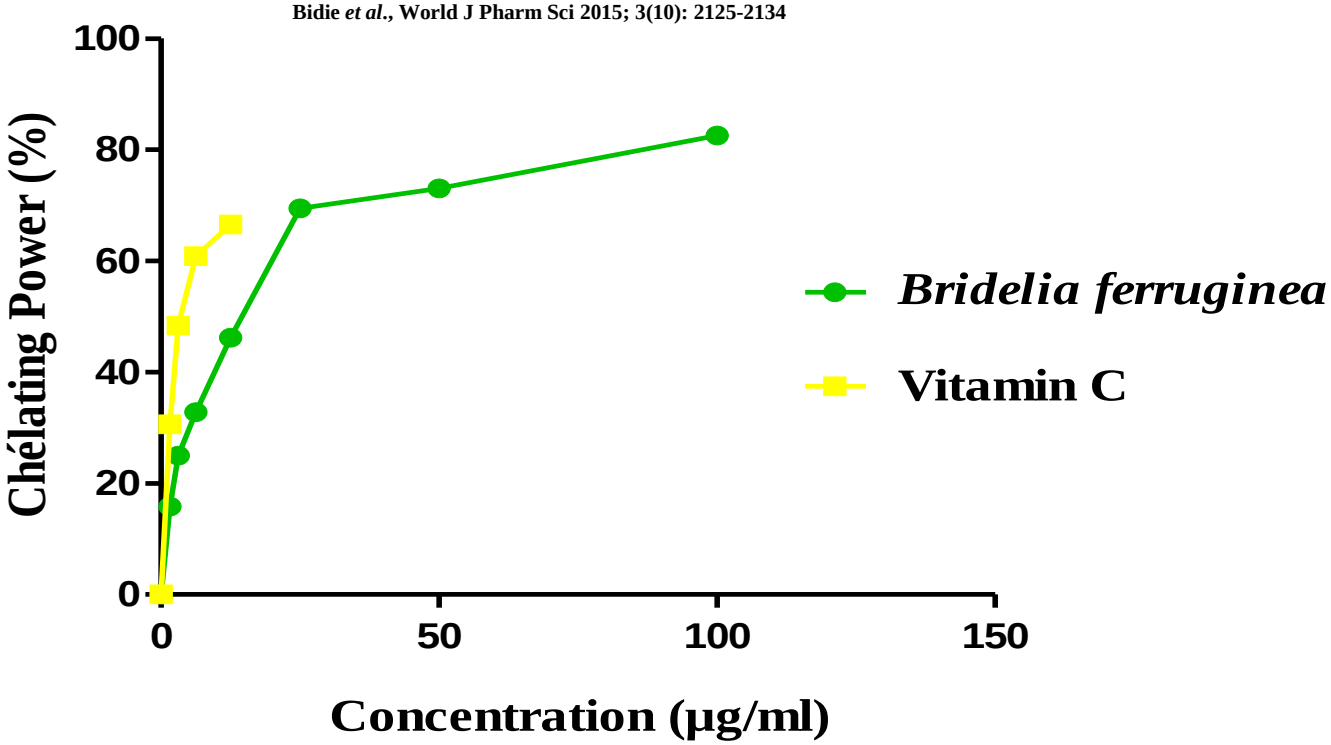


**Figure 5: Chelating power of plant extract in comparison of those of vitamin C**

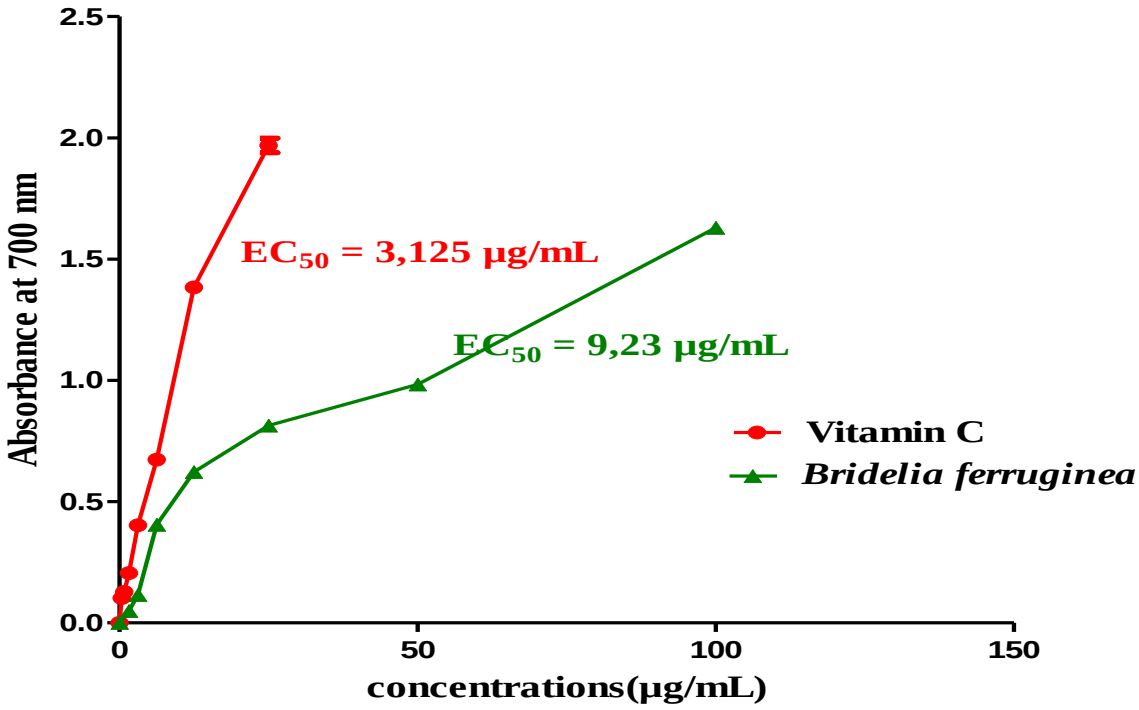


**Figure 6: Reducing power of *Brideliaferruginea* extract in comparison of those of vitamin C**

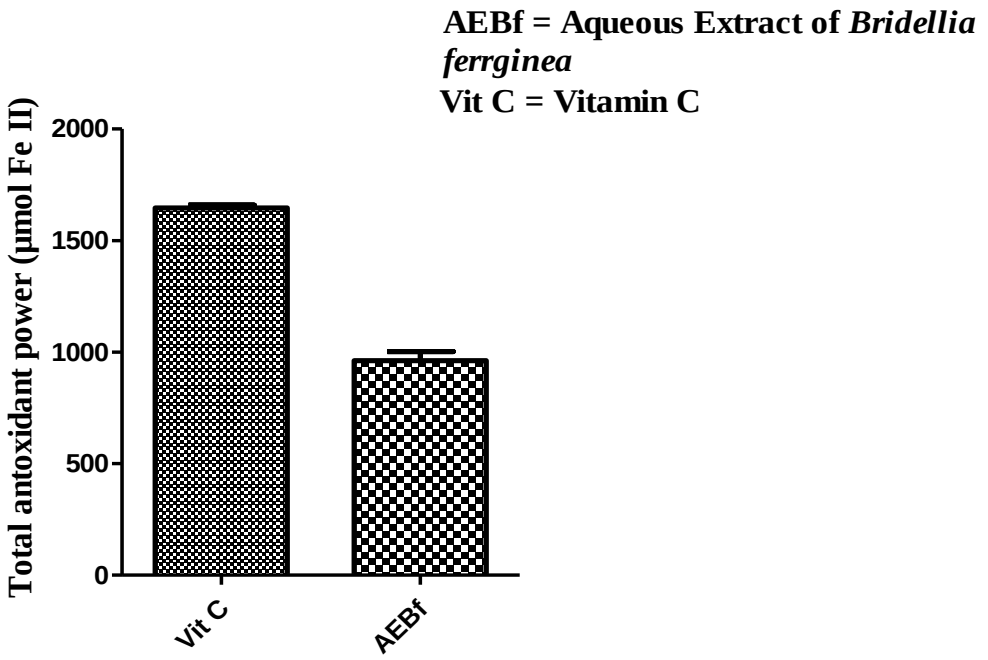


**Figure 7: Total antioxidant power of *Brideliaferruginea* in comparison of those of vitamin C**

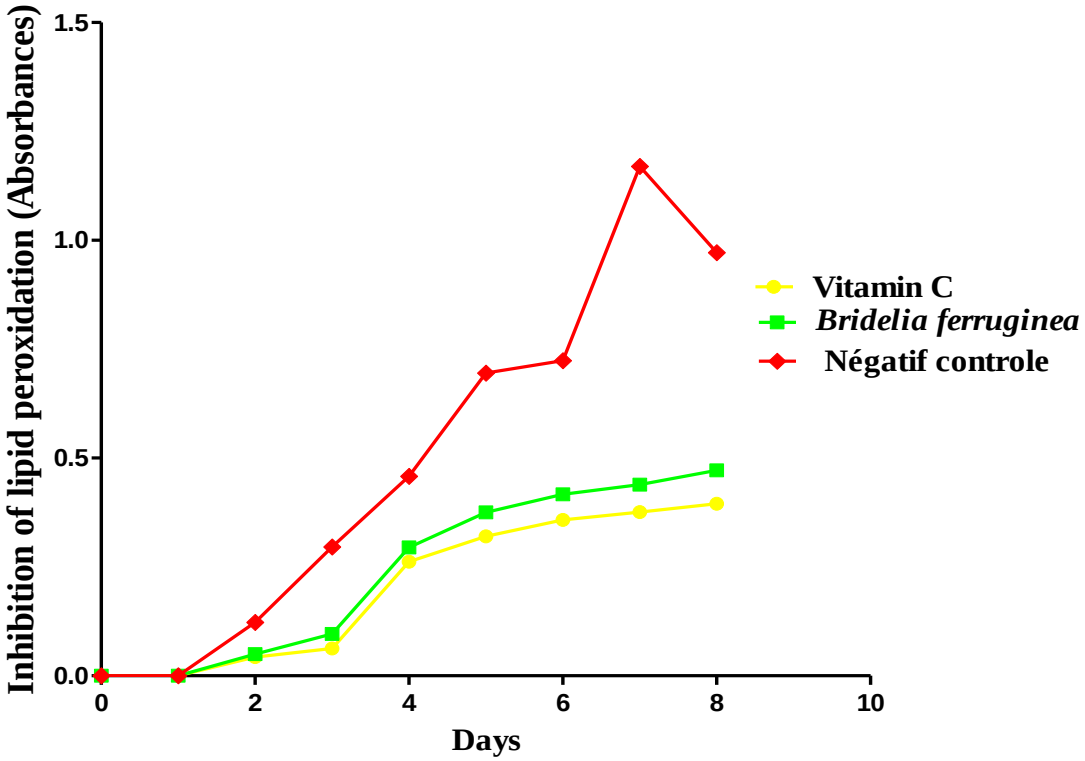


**Figure 8: Comparison of inhibition of lipid peroxidation by the method of FTC**

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