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Inhibitory Effects of Aqueous Extract of *Bridelia ferruginea* Stem Bark on Iron (II) Sulphate - Induced Oxidative Stress in Brain and Liver of Albino Wistar Rats

Adesola Oluwaseun Adeleye[1*], Omotade Ibidun Oloyede[2], Tolulope Oluwafemi Bolarinwa[1] and Oladejo Afees Adebayo[3]

[1]Department of Biochemistry, Federal University of Technology, Akure, Nigeria.
[2]Department of Biochemistry, Ekiti State University, Ado-Ekiti, Nigeria.
[3]Department of Chemical Science, Afe Babalola University, Ado-Ekiti, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author AOA designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author OIO wrote the protocol. Author TOB managed the analyses of the study. Author OAA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The antioxidant potential of *Bridelia ferruginea*, which is a widely used medicinal plant in Nigeria, sub-tropical Africa and parts of Asia was investigated using thiobarbituric acid reactive species (TBARS) assay. The aqueous extract of *Bridelia ferruginea* stem bark used in this study showed inhibition against the formation of thiobarbituric acid reactive species (TBARS) induced by Iron (II) sulphate in the liver and brain tissue homogenates of the locally bred male and female albino-Wistar rats used. The extract was discovered to have different antioxidant potentials in a manner that was concentration dependent. The aqueous extract of the plant showed a good percentage inhibition of 56.06% in the liver homogenate and 60.65% in the brain homogenate both at a concentration of 0.33 mg/ml using iron (II) sulphate as pro-oxidant with IC_{50} values of 3.00 ± 1.59

**Corresponding author: E-mail: adeseun4u2c@gmail.com;*

mg/ml for the liver and 3.00 ± 1.60 mg/ml for the brain. The result therefore suggests the medicinal benefit of *Bridelia ferruginea* in the treatment of various diseases induced by oxidative stress due to its ability to act as antioxidant.

Keywords: Bridelia ferruginea; antioxidant; oxidative stress; pro-oxidants.

1. INTRODUCTION

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage [1]. In humans, oxidative stress is thought to be involved in the development of many diseases or may exacerbate their symptoms. These include cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction, Schizophrenia, Bipolar disorder, fragile X syndrome, Sickle Cell Disease, and chronic fatigue syndrome [2].

Pro-oxidants are chemicals that induce oxidative stress, usually through either creating reactive oxygen species or inhibiting antioxidant systems. Reports have revealed that iron (II) sulphate act as a pro-oxidant by accelerating lipid peroxidation [3-5]. Iron (II) sulphate (or iron (II) sulfate, ferrous sulfate) is a chemical compound with the formula $FeSO_4$. It is used medically to treat iron deficiency, and also for industrial applications. All iron sulfates dissolve in water to give the same aquo complex $[Fe(H_2O)_6]^{2+}$, which has octahedral molecular geometry and is paramagnetic [3]. Iron (II) sulfate can be found in various states of hydration, and several of these forms exist in nature.

$FeSO_4 \cdot H_2O$ (mineral: szomolnokite, relatively rare)

$FeSO_4 \cdot 4H_2O$ (mineral: rozenite, white, relatively common, may be dehydratation product of melanterite)

$FeSO_4 \cdot 5H_2O$ (mineral: siderotil, relatively rare)

$FeSO_4 \cdot 6H_2O$ (mineral: ferrohexahydrite, relatively rare)

$FeSO_4 \cdot 7H_2O$ (mineral: melanterite, blue-green, relatively common).

All over the world, several hundreds of plants are good sources of medicinal agents and are used in traditional medicine for many different purposes, including bacterial and fungal infections. Traditionally, usage of plants in curing illnesses has deep roots in human history [6]. Ethno-pharmacological uses of plants prevail among the Nigerian people. It has been pointed out by Mukeshwar et al. [7] that plants continue to play a prominent role in primary healthcare of about 80% of the worlds' population. Many published reports have shown the effectiveness of traditional herbs against microorganisms. As a result, plants are one of the bedrocks for modern medicines to attain new principles [8].

Bridelia ferruginea (Family: Euphorbiaceae) is an indigenous medicinal plant in Nigeria. It is usually a gnarled shrub which sometimes reaches the size of a tree in suitable condition. Its common names in Nigeria include Kirni, Kizni (Hausa), Maren (Fulani), Iralodan (Yoruba), Ola (Igbo). Its habitat is the savannah. The bark is dark grey, rough and often marked scaly [9].

Bridelia ferruginea has diverse uses. The leaves have been used to treat diabetes. The plant is also used as a purgative and a vermifuge [10]. The bark extract is being used for milk coagulation and also in lime juice for the formulation of traditional gargle "ogunefu" [9]. Christian et al. [11] reported that the bark extract of the plant possess antimicrobial activities against some micro-organisms known to cause enteric and secondary upper respiratory tract infections, while Verma et al. [12] reported that the plant has anti-inflammatory activity. Owoseni et al. [13] reported the rich phytochemical content of its leaf and bark.

As oxidative stress appears to be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. Moreover, oxidative stress is both the cause and the consequence of disease. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. The objective of this research work is to determine the antioxidant potential of the stem bark of *Bridelia ferrugine*a plant using iron (II) sulphate as pro-oxidant.

Table 1. Phytochemical Analysis of *Bridelia ferruginea* Leaf and Bark [13]

Test	Bridelia leaf	Bridelia bark
Alkaloids	Present	Present
Flavonoids	Flavonoid present	Flavonoid present
	Catechol tannin suspected	Catechol tannin suspected
Tannin	Tannin confirmed	Tannin confirmed
	Taniferous confirmed	Taniferous confirmed
Cardiac glycosides	Deoxysugar present	Deoxysugar present
	Steroidal ring present	Steroidal ring present
	Steroidal nucleus present	Steroidal nucleus present
	Cardenoloids present	Cardenoloids present
Anthraquinone	Free anthraquinone present	Free anthraquinone present
	Bound anthraquinone present	Bound anthraquinone present
Phlobatinnins	Present	Present
Saponin	Saponin confirmed	Saponin confirmed
Cyanogenic glycosides	Anthocyanin absent/negative	Anthocyanin absent/negative

2. MATERIALS AND METHODS

2.1 Preparation of Plant Extract

The bark of *Bridelia ferruginea* was collected from the Okesa market, Ado-Ekiti, and authenticated in the Department of Plant Science, Ekiti State University, Ado-Ekiti, by a botanist with herbarium code number UHAE 46. 1g of the bark was pounded and soaked in 100 ml of distilled water for 24 hr. The extract was filtered using a Whatman filter paper and kept under a cool temperature until further analysis.

2.2 Animal Preparation

All animal procedures were in strict accordance with the NIH guide for the care and use of laboratory animals. 21 locally bred male and female albino-Wistar rats with an average weight of 200±20g, fed on standard diet and allowed water *ad libitum* were used for *In vitro* studies. The animals were grouped according to their gender and housed (two rats per cage) in a well-ventilated room.

2.3 Production of Thiobarbituric Acid Reactive Species (TBARS) from Animal Tissues

The rats were sacrificed with cervical dislocation. The liver and brain tissues were quickly removed and placed on ice. 1 g of the tissues were homogenized in cold 0.1M Tris-HCL buffer pH 7.4 (1: 10 w/v) with about ten up and down strokes at approximately 1200 rpm in a Teflon glass homogenizer.

The homogenates were centrifuged for 10 min at 3000 rpm to yield a pellet that was discarded and the supernatants were used for the assay.

Production of thiobarbituric acid reactive species (TBARS) was determined using a modified method of Ohkawa et al. [14] as described by Puntel et al. [15].

2.4 Statistical Analysis

Data were analyzed statistically by one way ANOVA, followed by Duncan's multiple range test (DMRT) where appropriate. Statistical comparisons were performed with student's t-test. Differences were considered significantly at $p<.05$. The correlation coefficient (R^2) between the parameters tested was established by regression analysis.

3. RESULTS AND DISCUSSION

Iron is an essential element for normal cellular physiology but excess iron in the body can cause cell injury [16]. This is because of the catalytic role it plays in initiation of free radical reactions. The resulting radicals have the potential to damage cellular lipids, nucleic acids, proteins and carbohydrates, this result in wide-ranging impairment in cellular function and integrity. The mechanism by which iron can cause this deleterious effect is that Fe(II) can participate in Fenton reaction, reacting with hydrogen peroxide (H_2O_2) to produce hydroxyl radical (OH^-) [17]. Increase in the formation of thiobarbituric acid reactive species (TBARS) in iron (II) sulphate induced oxidative stress as compared to the normal suggests possible damage of tissue

where an overload of iron in the cytosol and mitochondrial can cause considerable oxidative damage by increasing superoxide production which can react with Fe (III) that participates in Fenton's reaction [16]. Iron overload results in cellular damage in a number of tissue including liver and brain which are used in this research work. Storage of iron in the liver leads to liver cirrhosis. The possible mechanism of iron toxicity includes free radical mediated peroxidation which is readily catalyzed by iron [18].

Table 2 shows the inhibitory effect of aqueous extract of *Bridelia ferruginea* bark on Iron (II) sulphate induced lipid peroxidation in a rat liver homogenate. The result shows an increase in the formation of thiobarbituric acid reactive species (TBARS) in Fe^{2+} induced oxidative stress of control (0.950 ± 0.117) when compared with the normal/basal (0.611 ± 0.256). Aqueous extract of *Bridelia ferruginea* significantly reduced the accumulation of lipid peroxides in a concentration dependent manner. There was no significant difference between the absorbance at different concentrations when compared to the basal. The highest inhibition against iron (II) sulphate lipid peroxidation was observed at a concentration of 0.33mg/ml with 56.36 ± 6.49% inhibition when compared to other concentrations. Meanwhile, a concentration of 3.33mg/ml produced the lowest inhibition at 20.43 ± 15.29%.

The inhibitory effect of aqueous extract of *Bridelia ferruginea* stem bark on iron (II) sulphate ($FeSO_4$) induced lipid peroxidation in a rat brain homogenate is shown in Table 3. There was also a statistically significant (P<.05) increase in the absorbance of control (0.667 ± 0.123) i.e. in the formation of TBARS, when compared with the basal/normal brain homogenate (0.405 ± 0.022). There was no significant difference among the absorbance at concentrations 0.33 – 2.67 mg/ml when compared with the basal. Also, different concentrations of aqueous extract of *Bridelia ferruginea* (0.33 - 3.33 mg/ml) shows a significant inhibition of iron (II) sulphate induced lipid peroxidation in rat brain homogenate in a concentration dependent manner as seen in Table 3. The plant shows the highest percentage inhibition of 60.65 ± 4.01% at a concentration of 0.33 mg/ml and the lowest percentage inhibition of 6.00 ± 4.77% at a concentration of 3.33 mg/ml.

Table 2. The inhibitory effect of aqueous extract of Bridelia ferruginea stem bark on iron (II) sulphate induced lipid peroxidation in a rat liver homogenate

Concentration (mg/ml)	Absorbance (532 nm)	% Inhibition	Linear Equation (R^2)	IC_{50}(mg/ml)
Basal	0.611 ± 0.256^{bcd}	-	y = -8.919x + 63.83	3.00±1.59
Control	0.950 ± 0.117^{a}	-		
0.33	0.412 ± 0.017^{d}	56.06 ± 6.49	R^2 = 0.985	
0.67	0.502 ± 0.027^{cd}	46.36 ± 8.66		
1.33	0.615 ± 0.075^{bcd}	34.13 ± 14.32		
2.67	0.669 ± 0.053^{bc}	28.43 ± 13.48		
3.33	0.750 ± 0.062^{ab}	20.43 ± 15.29		

Results are expressed as mean of three experiments ± SD
Values with different notations are significantly different (P < .05)

Table 3. The inhibitory effect of aqueous extract of *Bridelia ferruginea* stem bark on iron (II) sulphate induced lipid peroxidation in a rat brain homogenate

Concentration (mg/ml)	Absorbance (532 nm)	% Inhibition	Linear Equation (R^2)	IC_{50} (mg/ml)
Basal	0.405 ± 0.022^{cd}	-	y = -12.86x + 71.40	3.00±1.60
Control	0.667 ± 0.123^{a}	-		
0.33	0.265 ± 0.075^{d}	60.65 ± 4.01	R^2 = 0.978	
0.67	0.393 ± 0.066^{cd}	40.80 ± 3.81		
1.33	0.441 ± 0.147^{bcd}	35.16 ± 12.14		
2.67	0.532 ± 0.163^{abc}	21.46 ± 12.62		
3.33	0.622 ± 0.085^{ab}	6.00 ± 4.77		

Results are expressed as mean of three experiments ± SD
Values with different notations are significantly different (P < .05)

The plant generally shows almost the same ability to inhibit thiobarbituric acid reactive species (TBARS) production in both liver and brain tissues using iron (II) sulphate as pro-oxidant because the IC_{50}, which is the concentration of the plant extract capable of bringing about 50% inhibition of iron (II) sulphate induced lipid peroxidation, of the liver (3.00 ± 1.59 mg/ml) and that of the brain (3.00 ± 1.60 mg/ml) are significantly same (Tables 2 & 3 respectively). The strong inhibitory effect of *Bridelia ferruginea* was shown by its ability to protect against the formation of thiobarbituric acid reactive species (TBARS) in the liver and brain respectively. This tremendous activity may be as a result of the great phenolic content of this plant as suggested by Jittawan and Sirthon [17]. Because of the phenols like flavonoids and bioflavonoids, *Bridelia ferruginea* has been observed to be a very potent chelator of iron in its mg/ml quantity and this will help to forestall the formation of lipid peroxidation in organs (liver and brain) [17].

4. CONCLUSION

The protection offered to the liver and brain tissues by the aqueous extract of *Bridelia ferruginea* stem bark in this research, with regard to its inhibitory effect against oxidative stress, therefore suggests that they may be useful in the treatment of liver and brain diseases resulting from iron overload.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

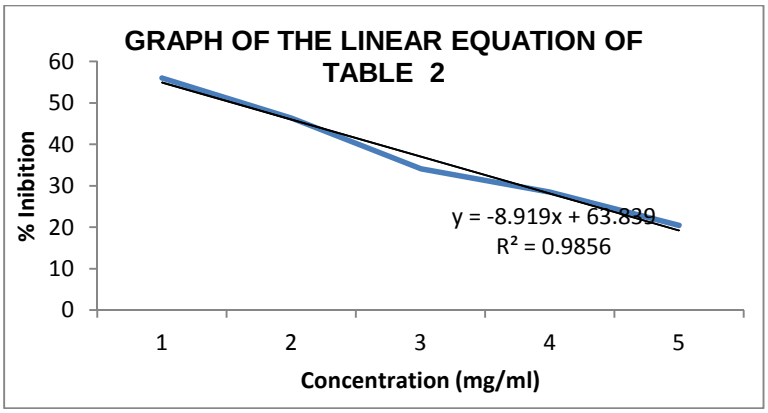
Appendix 1: Statistical Analysis

$$\text{Mean} = \frac{\sum x}{N}$$

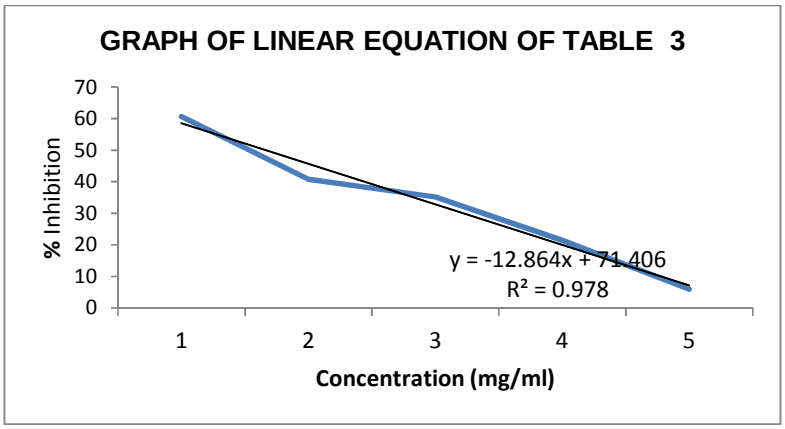
$$\text{Standard deviation} = \sqrt{\frac{\sum (x - \bar{x})^2}{N - 1}}$$

$$\text{Percent (\%) inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of Test}}{\text{Absorbance of control}} \times 100$$

Appendix 2.



Appendix 3.



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