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# INVITRO ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF BRIDELIA FERRUGINEA (STEM BARK)

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## ABSTRACT

*The antioxidative and neuroprotective potential of Brideliaferruginea, a widely medicinal plant in sub-tropical Africa and part of Asia were investigated. The ethanolic extract of Bridelliaferruginea bark in this study showed inhibition against the formation of thiobarbituric acid reactive species (TBARS ) induced by iron (II) sulphate (60µM $FeSO_4$) in the brain and liver homogenates of the albino rat used. The extract was found to have different antioxidant potentials in a manner that was concentration dependent, the result showed 36.9µg/ml of the extract to have the most potent inhibition at 54.16% and 8.46% for the brain and liver respectively. The result of the present study suggests the potential use of Brideliaferruginea in the treatment of various diseases. Iron chelation procedures also support the results obtained from thiobarbituric acid reactive species (TBARS), as this plant proved to be an effective iron chelator. Further studies would be important to determine whether using other solvent as a vehicle would produce different effects.*

**Keywords:**Thiobarbituric acid reactive species; Brideliaferruginea ; iron chelation

## INTRODUCTION

Over the last two decades, while the health of the advanced nations has tremendously improved, many poor nations, particularly those in Africa and Asia, have experienced health decline. In addition to HIV/AIDS and the perennial problems of infectious diseases (e.g. malaria, helminthiasis and cholera), many African countries are also witnessing a huge rise in conditions such as diabetes, hypertension and cancer. A recent report in one of the leading newspapers in Nigeria highlighted the growing problem of diabetes in the country with doctors expressing grave concern about the rate of increase in diabetic cases. The report noted that apart from genetic predisposition and environmental issues, the lifestyle of Nigerians was the major cause of the upsurge (Asare, 2005). A consultant of the hospital specifically noted the eating habits (a taste for refined sugary foods instead of fruits and vegetables), lifestyle (sedentary jobs with less exercising) and cultural practices especially where obesity was cherished and considered as a sign of affluence as the predisposing factors, also exposure to cigarette smoke, environmental pollutants such as emission from automobiles and industries, consumption of alcohol in excess, asbestos, exposure to ionizing radiation, and bacterial, fungal or viral infections all these result in the generation of reactive oxygen species which initiate these diseases.

Research efforts must be directed towards finding cost effective solutions to such debilitating disorders, there would be long term repercussions. Part of this solution lies in the arsenal of medicinal plants. These plants have antioxidant properties that help to prevent lipid peroxidation which initiate some of these diseases. This research therefore highlights the therapeutic potential of one such plant belonging to the genus Bridelia that can be used to fight these different degenerative diseases. The genus Bridelia consists of about 60 species including Brideliaatroviridis, Brideliacathartica, Brideliaferruginea, Brideliamicrantha, Brideliaovata, Brideliasiamensis, Brideliatomentosa and Brideliatulasneama, all of which are native to Africa, Asia and Australia (Rashid, 2000). Brideliaferruginea, belongs to the family Euphorbiaceae. Among its many Nigerian vernacular names are opamfufuo, baree (Twi); flatsho (Ga-Dangme); ekpazenra (Nzema); and kirni (Hausa),Epoira (Yoruba) Brideliaferruginea (Euphorbiaceae) is a subtropical medicinal plant widely used in traditional African medicine for the treatment of conditions such as rheumatic pains, headaches, gastrointestinal and urogenitaldisorders (Addae-Mensah, 1992; Akinpelumi and Olorunmola, 2000 ). The Hausa people of the West African sub region believe that prophylactic use of the root

decoction can prevent syphilis.Ithas also been reported an application of the expressed bark is an effective antidote for the wound inflicted by arrow poison. Such wounds are treated by applying the chewed bark to the affected area followed by sucking. Its diuretic action has also been found helpful in the treatment of gonorrhea (Addae-Mensah, 1992).

Brideliaferruginea has a great antioxidant potential which can be used to protect the body against damage caused by free radicals which is regularly produced in vivo and oxidative stress induced by these free radicals. In this study, the relationship between health-promoting pharmacological and antioxidant effects of the ethanolic extract of Brideliaferruginea is evaluated.

## MATERIALS AND METHODS

### Preparation of Plant Extract

The bark of Brideliaferruginea was collected from Oye-Ekiti, Ekiti state, Nigeria in October,2010. It was then authenticated by Mr. Omotayo, a plant scientist at the University of Ado Ekiti, Ekiti state, Nigeria. Voucher specimen was deposited at the herbarium of the faculty of science of the university. The powered bark was extracted with cold 70% ethanol at room temperature ($27^0$C) for 48 hours. The filtrate (ethanolic extract) was evaporated to dryness at $60^0$c using rotary evaporator, the yield was found about 19.67%.

### Test Animals

All animal procedures were in strict accordance with the NIH Guide for the care and use of laboratory animals. Two to three month old wistar rats (200-250g) were used for the in vitro studies.

### Production of Thiobarbibutric Acid Reactions Species (TBARS)

The lipid peroxidation assay was carried out using the method described by Ohkawa et al., 1979 as described by Puntel et al., 2005.The rats were killed by cervical dislocation. Liver and brain tissues were quickly removed and placed on ice one gram of tissues was homogenized in cold 0.1M tris buffer $p^H$ 7.4(1:10w/v) up and down in a Teflon homogenizer. The homogenates were centrifuged for 10 min at 3000g to yield a pellet that was discarded and the supernatant was used for the assay. The supernantant with or without 50µl of the freshly prepared pro-oxidant (iron (II) sulfate), different concentrations of the plant extracts and an appropriate volume of distilled water whicch gives a total volume of 300 µl were incubated at $37^0$c for I hour. The colour reaction was carried out by adding 200,500 and 500 µl each of the 8.1%sodium dodecyl sulphate (SDS),1.33M acetic acid($p^H$ 3.4) and 0.6%TBA respectively. The reaction mixture including those of serial dilution of 0.03mM standard MDA were incubated at $97^0$c for 1hr.the absorbance was read after cooling at a wavelength of 532nm in a vis/uv spectrophotometer.

### Iron Chelation Assay

The ability of the ethanolic extract of Brideliaferruginea to chelate $Fe^{2+}$ was determined using a modified method described by Puntel et al (2005). Briefly,150µl of freshly prepared 1mM $Feso_4$ was added to a reaction mixture containing 168ul of 0.1M Tris-Hcl(pH 7.4),218µl saline(0.9%)Nacl and the ethanolic extract of Brideliaferruginea (1-10µg/ml).The reaction mixture was then incubated for 5mins, before adding 13µl of 0.25%1,10-phenanthroline(w/v).The absorbance was subsequently measured at 510nm in the spectrophotometer

### Statistical Analysis

The data were analyzed statistically by one way ANOVA followed by Duncan multiple range test when appropriate.

## RESULTS AND DISCUSSION

Oxidative stress is considered to be associated with a number of diseases. It is has also been implicated in the normal aging process.(Gems and Patridge, 2008).There also exists a strong correlation between thiobarbituric acid reactive species (TBARS) as a marker of lipid peroxidation and products that reflect oxidative damage to DNA. Iron is an essential element for normal cellular physiology but

excess iron in the body cause cell injury. 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 acid, protein and carbohydrates, this results 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($0H^*$) (Fraga and Oteiza, 2002; Harris et al., 1992Increase in the formation of TBARS in Iron

(II) sulfate induced oxidative stress, as compared to the normal suggests possible damage of tissue where an over load of Iron in the cytosol and mitochondria can cause considerable oxidative damage by increasing superoxide production which can react with Fe(III) that participates in the Fenton's reaction(Fraga and Oteiza,2002; Harris et al.,1992). Iron overload results 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 (Jittawan , 2008).

The protection offered by ethanolic extract of Brideliaferruginea suggests that they may be useful in the treatment of liver and brain diseases resulting from this overload. The human body is equipped with antioxidant defense system that deactivates these highly reactive free radicals. Antioxidant enzymes made in the body and antioxidant nutrient(found in foods) soak up all the energy that these free radicals have, turning them into harmless particles that can be metabolized, so these antioxidants are functional components of that have extra health benefits in the body(Oboh et al., 2007). Phenols including flavoniods, can protect the body cell against the damage caused by reactive oxygen species (ROS).Much of the potentials of Brideliaferruginea is associated with the phenolic content of this medicinal plant.

Many recent research reports confirm that flavonoids are even more potent antioxidants than vitamin C and vitamins E. The resulting peoxylradical produced from lipid peroxidation chain reaction have the potentials of damaging cellular lipids, nucleic acids, proteins and carbohydrate; the result is wide ranging impairments in the function and integrity (Britton et al., 2002). Iron(II)sulfate was used to induce lipid peroxidation for the purpose of this research work because Fe(II) can react with hydrogen peroxide($H_20_2$) to produce the hydroxyl radical(OH) via Fenton reaction whereas, superoxide can react with Iron(III) to generate Iron(II)that can participate in Fenton reaction(Fraga and oteiza 2002; Harris et al., 2002).The over production of reactive oxygen species can directly attack the polyunsaturated Fatty acids of the cell membrane and induce lipid peroxidation.

The effect of free radicals in the brain has been well studied and it is concluded that there is high cell degradation in the brain. The brain and nervous system are particularly vulnerable to oxidative stress as a result of a limited antioxidant capacity caused by its limited access to antioxidants produced in the body .Though the brain is about 2% of the entire body weight, It uses 20% of the oxygen produced in the body. The brain cannot make glutathione. It relies on the surrounding astrocyte cells to produce usable glutathione. Neurons are the first cells to be affected by shortage of antioxidant and are mostly susceptible to oxidative stress. Moreover, the chelating ability of iron is used in this research work to as an indicator of the protective potentials against diseases, because Iron is involved in the pathogenesis of Alzheimer's disease and other disease by multiple mechanisms (Elise and James, 2002).

Brideliaferruginea is discovered to have an effective TBARS inhibiting ability in the liver and brain because its antioxidant potentials at different concentrations ranging between 3.3 µg/ml-39.6µg/ml produced inhibition in its µg quantity. The ethanol used as a solvent has no antioxidant potentials as it produced little or no inhibition and sometimes a negative value. In most cases, there was an increased ability of the ethanolic extract to reduce TBARS production to less than normal level as shown in table 3.1 and 3.2 for the liver and brain respectively. The highest inhibition was observed with the 10µg/ml concentration in the both organs, with that of the liver being exceptionally high. Meanwhile, 3.3µg/ml produced the lowest inhibition in both organs with the lower coming from the brain. 6.6µg/ml has almost the same effect on both organs and are fairly protective against the formation of TBARS and while the inhibition increased in the 13.2µg/ml in liver, sharp decline was observed at the same concentration in the brain while the 26.4µg/ml concentration produced a decreased inhibition in both

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organs with that of the brain being fairly high when compared with the control. 3.3µg/ml has a reduced inhibitory effect against TBARS produced in both liver and the brain with that of the brain lower than the result obtained from the activity is very high at the 3.26µg/ml concentration and the plant generally shows a high ability to inhibit TBARS production more in the liver, as a higher potency ratio is observed in all the concentration in the liver when compared side by side with the brain's result. The IC50 of the brain (13.38µg/ml) also confirmed this as the IC50 is only 3.851µg/ml. All the concentration however produced a high inhibition when compared with control and the basal but the effectiveness is observed the highest at the 10µg/ml in both tissues and the various effects have been confirmed to be concentration dependent.

The use of iron chelation as a measure of the management of Fe (II) associated oxidative stress in the brain and liver. If the iron is chelated, it will be unable to form complexes with free radicals hence lipid peroxidation will not be initiated. Different concentration were evaluated and indicated in fig 3.1. The different concentrations from stock proved to be very potent in its milgram quantity. This result showed the 2µg/ml to be the most potent and very effective against iron .i.e. it can greatly chelate iron when compared with the control. After an increase in potency by 1µg/ml., there was a steady decrease in the potency and this also witness an upsurge in rise with the 10µg/ml of the dilute fraction while the stock solution results were all exceptionally high with above 85% inhibition and the different concentration (mg/ml) all produced slight differences.

Neuroprotective activities of Brideliaferruginea was shown by the ability to chelate iron and protect against the formation of Thiobarbitum reactive species.(TBARS) the liver and brain respectively. This tremendious activity may be as a result of the great phenolic content of this plant as suggested by (Jittawan and Sirthon, 2008). Because these phenols like flavonoids and biflavonoids, Brideliaferruginea has therefore been observed to be a very potent chelator of iron in its µg/ml quantity and this will help to forestall the formation of lipid peroxidation in these organs.

This study revealed that ethanolic extract of Brideliaferruginea bark prevents iron (II) sulfate induced lipid peroxidation in both the liver and the brain in vitro. However the 39.6µg/ml has the highest potency as it produced the highest inhibition in both tissues. The plant also showed a good iron chelating ability at all the concentration used. Its toxicity to human has not yet been reported.

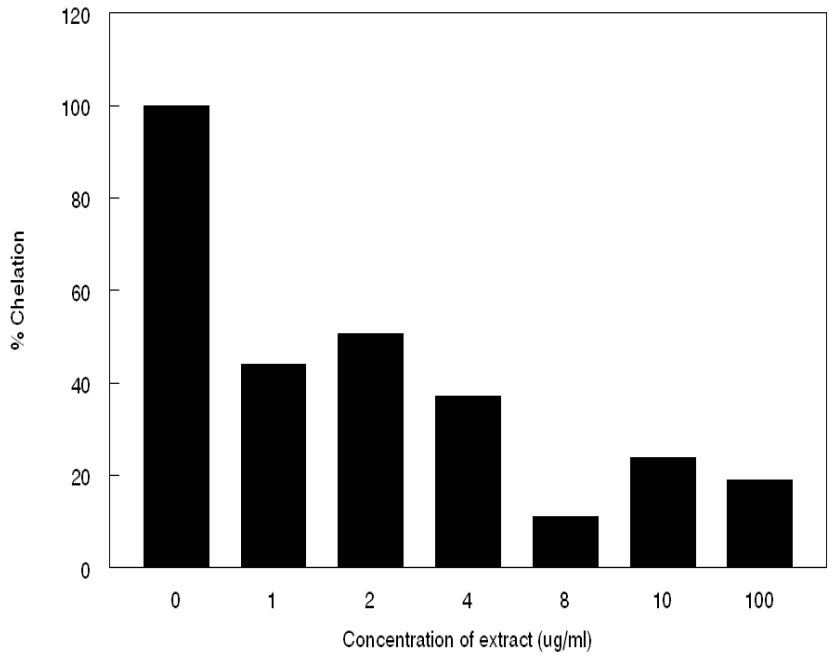


Figure 1.Iron Chelating ability of Ethanolic Extract of Brideliaferruginea( stem bark)

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Table 1.The inhibitory effect of ethanolic extract of Brideliaferruginea on iron (II) sulfate-induced lipid peroxidation in rat brain homogenate.

| *Conc (µg/ml)* | *MDA(nmole/mg tissue)* | *% inhibition* | *Logarithm equation* | *$IC_{50}$(ug/ml)* |
|---|---|---|---|---|
| Normal | 0.07±0.28 | | | 13.38±36.68 |
| Control | 0.53±0.12 | | y=8.3146In(x)+25.15 | |
| Solvent | 0.50±0.06 | -2.25±29.90 | $r^2$=.01435 | |
| 3.3 | 0.38±0.12 | 10.51±21.05 | | |
| 6.6 | 0.29±0.75 | 48.33±16.88 | | |
| 13.2 | 0.21±0.15 | 44.01±16.72 | | |
| 26.4 | 0.25±0.21 | 34.12±12.14 | | |
| 33.00 | 0.26±0.16 | 30.88±20.16 | | |
| 39.6 | 0.14±0.11 | 54.16±22.86 | | |
| 326 | 0.39±0.19 | 28.71±24.01 | | |

Results are expressed as means of three experiments in duplicates ± standard deviation

Table 2 .The inhibitory effect of ethanolic extract of Brideliaferruginea on iron (II) sulfate-induced lipid peroxidation in rat liver homogenate

| *Conc (µg/ml)* | *MDA (nmole/g tissue)* | *% inhibition* | *Logarithm equation ($r^2$)* | *$IC_{50}$ (ug/ml)* |
|---|---|---|---|---|
| Normal | 0.35±0.48 | | | |
| Control | 0.43±0.02 | | | |
| Solvent | 0.43±0.11 | 20.84±09.1 | y=26.065In(x) | $IC_{50}$ |
| 3.3 | 0.41±0.11 | 28.47±12.4 | $r^2$=0.6354 | |
| 6.6 | 0.23±0.06 | 48.81±05.18 | | |
| 13.2 | 0.25±0.06 | 62.72±23.3 | | |
| 26.4 | 0.31±0.22 | 55.31±32.75 | | |
| 33.00 | 0.31±0.79 | 43.15±17.94 | | |
| 39.6 | 0.21±0.10 | 82.46±07.14 | | |
| 326 | 0.35±0.88 | 92.82±25.86 | | |

Results are expressed as means of three experiments in duplicates ± standard deviation.

## CONCLUSIONS

Brideliaferruginea bark has proved to be a cheap, affordable and equally potent therapeutic plant. Its use as a functional food should be promoted as it shows effectiveness in the treatment of different diseases such as cancer and liver diseases among others.

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