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***J. Nat. Prod. Plant Resour*., 2011, 1 (4): 90-95**
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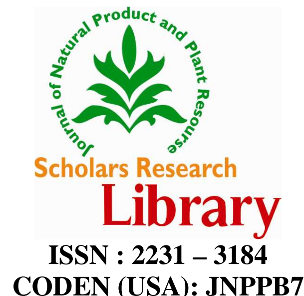

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Antioxidant potential of *Swertia quartiniana*

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ABSTRACT

Antioxidant potential of ethanolic extract of Swertia quartiniana leaves was determined using thiobarbituric acid reactive species (TBARS) and iron chelation assays. The result of the study revealed that incubating rat's brain and liver homogenates in the presence of 60µMFe^{2+} caused increased in malondaldehyde (MDA) production in rat's brain and liver homogenate when compared with normal. However the ethanolic extract of Swertia quartiniana leaves caused decrease in MDA content in both brain and liver homogenates. The extract had higher inhibitory effect at concentration of 29µg/ml ($1c_{50}$ 2.97µg/ml) in the liver and at concentration of 44µg/ml ($1c_{50}$ 0.11µg/ml) in the brain. The extract also showed chelating ability of 76% at concentration of 110µg/ml. The inhibitory effect of the ethanolic extract of Swertia quartiniana using both assays were concentration dependent. It was therefore concluded that ethanolic extract of Swertia quartiniana prevent Fe^{2+} -induced lipid peroxidation due to higher Fe^{2+} chelating ability.

Keyword *Swertia quartiniana,* malondaldehyde, thiobarbituric acid reactive species, iron chelation.

INTRODUCTION

Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn these free radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions, they do this by being oxidized themselves, so antioxidant are often reducing agents such as ascorbic acid thiols or polyphenol [1].

Although oxidation reactions are crucial for life, they can also be damaging, hence plants and animals maintain complex system of multiple types of antioxidants such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidase. Antioxidant metabolites and enzymes work to prevent oxidative damage to cellular components such as proteins, lipids and DNA by either preventing these reactive species (free radicals) from being formed or remove them before they can damage vital component of the cell []. Iron is an essential metal for normal cellular physiology,but excess iron can result in cell injury. This is because it plays a catalytic role in the initiation of free radical reactions.The resulting oxyradicals have the potential to damage cellular lipids, nucleic acids, proteins and carbohydrates; the result is wide-ranging impairment in cellular function and integrity [3].

Some compounds contribute to antioxidant defense by chelating transition metal and preventing them from catalysing the production of free radicals in the cell. Free radicals are atoms or groups of atoms that have one or more unpaired electrons which make the unstable. They are reactive molecules and can react with proteins, nucleic acid lipid and other molecules to alter their structure and produce tissue damage[4]. Oxidative stress occur when the production of reactive oxygen species is greater than the body's ability to neutralise or eliminate them. Foodstuffs supply not only energy, essential amino acids, fiber, vitamins, and minerals but also some active compounds such as antioxidants (tocopherols, carotenoids, vitamin C, phenolic compounds, etc.) that may have different beneficial functions in thebody. Dietary components, which are capable of acting as antioxidants, are likely to be beneficial by augmenting cellular defenses and protecting the cell against damage caused by free radicals, by acting as radical scavengers, reducing agents, potential complexes of prooxidant metals, and quenchers of singlet oxygen formation [4-8].

a plant of Gentianaceae family. It is a four angled plant having opposite leaves with their apex acuminate. *Swertia quartiniana* is perennial diffused herbs with weak stem having white or purple flower. The fruits are capsule in nature, it is usually cultivated. *Swertia quartianiana* is distributed in West Africa Ethiopia, East and South tropical Africa [9]

Swertia quartiniana is a medicinal plant that has been used locally in the treatment of diseases like malaria, hypertension and diabetes but not yet documented. The aim of the study is to determine the antioxidant potential of *Swertia quartiniana* which might be attributed to its use as medicinal plant.

MATERIALS AND METHODS

2.1 Materials

Thiobarbituric acid (TBA) malonaldehydebisdimethylacetal (MDA) sodium dodecylsulphate (SDS), acetic acid, ethanol, Tris HCl, O- phenanthroline and iron II sulphate.

2.2 Preparation of plant extract

The leaves were collected in October 2010 from Iloro in Ijero Local Government, Ekiti State and authenticated by Mr. Felix Omotayo from Faculty of Science University of Ado-Ekiti. The leaves were extracted with cold 75% ethanol with at room

temperature(27^{0}c) for 48hours. The filtrate (ethanolic extract) was evaporated to dryness at 60^{0}c in a water bath giving a percent yield of 15% .

2.3 Test Animals

All animal procedures were in strict accordance with the NHI Guide for the care and use of laboratory animals. Two to three months old albino rats (200-250g) were used for invitro studies.

2.4. Production of thiobarbituric acid reactive species (TBARS)

Production of TBARS was determined using a modified method of [10] as described by [11] the rats were sacrificed by cervical dislocation. Liver and brain tissues were quickly removed and placed on ice. One gram of tissue was homogenized in 0.1MTris buffer pH 7.4(1:10w/v) in up and down Teflon homogenizer. The homogenates were centrifuged for 10minutes at 3000revolution per minute to yield a pellet that was discarded and the supernantant was used for the assay. The supernatant (100µl)with or without 50µl of freshly prepared pro-oxidant(Iron II sulphate),different concentrations of the plant extract and appropriate volume of distilled water to give a total volume of 300µl were incubated at at 37^{0}c for 1hour. The colour reaction was carried out by adding 200, 500 and 500µl each of the 8.1% sodium dodecyl sulphate (SDS) acetic acid (pH 3.4) and 0.6% thiobarbituric acid (TBA) respectively. The reaction mixtures including those of serial dilution of 0.03mM standard MDA were incubation at 97^{0}c for 1hour. The absorbance was read after cooling at a wave length of 532nm in a visible/uv spectrophotometer.

2.5 Iron Chelation Assay

The ability of the ethanolic extract of *Swertia quartiniana* to chelate Fe (II) was determined using a modified method of [11]. Briefly 20µl of freshly prepared 1mM $FeSO_4$ were added to a reaction mixture containing 168µg of 0.1M Tris HCl (pH 7.4), 218µl saline(0.9% Nacl) and the ethanolic extract of the plant the reaction mixture was left for 5minutes before the addition of 13µl of 0.25% 1, 10-phenanthroline (w/v).The absorbance was subsequently measured at 510m in the spectrophotometer.

2.6 Statistical Analysis

The result was expressed as mean ± standard deviation. Data were analyzed statistically by one-way ANOVA, followed by Duncan's multiple range test when appropriate and regression analysis.

RESULTS AND DISCUSSION

Degenerative diseases and aging processes associated with Fe accumulation could be managed prevented by Fe chelators [1 ,13].

Oxidative stress occurs as a result of overproduction of reactive oxygen species which the body cannot neutralise or eliminate from the body. Oxidative stress is thought to contribute to the development of wide range of diseases e.g. cardiovascular, diabetes, neurodegenerative disease, pancreatic atrophy etc. Lipid peroxidation of membrane is a possible mechanism of red cell membrane in a sickle cell patient relative to normal also neurodegenerative disease and aging

processes associated with iron accumulation could be managed and prevented by iron chelator [1]. One of the ways polyphenol exhibit their antioxidant activity is by chelating iron. The result of this study revealed that ethanolic extract of *Swertia quartiniana* inhibit lipid peroxidation in a concentration dependent manner in rat's brain and liver homogenates as shown in Tables 1 and 2 respectively. However, ethanolic extract of *Swertia quartiniana* leaves had higher inhibitory effect on lipid peroxidation in rat's brain and liver especially at concentration of 29µg/ml and had minimum inhibitory effect at concentration of 3.7µg/ml. No inhibitory effect was observed at extract concentrations 37µg/ml, 44µg/ml and 367µg/ml respectively in liver homogenate.

In the brain homogenate the ethanolic extract had maximum inhibitory effect at concentration of 44µg/ml and minimum inhibitory effect at concentration of 37µg/ml. The protective action of ethanolic extract of *Swertia quartiniana* leaves could be attributed to the presence of ascorbic acid and phenol [14]. Incubation of rat's brain and liver homogenates in the presence of 60µM Fe^{2+} caused increase in MDA content when compared with normal. Malondaldehyde (MDA) is a product of lipid peroxidation induced by iron The increase in MDA content that results from lipid peroxidation in the presence of iron could be attributed to the fact that iron (Fe^{2+}) can analyze one electron transfer reaction that generate reactive oxygen species such as the reactive OH which is formed from H_2O_2 (Hydrogen peroxide) through the Fenton reaction. Iron also decomposes lipid peroxides thus generating peroxyl and alkoxyl radical which favour the propagation of lipid peroxidation [15] which damage cell membrane lipid. The ethanolic extract of *Swertia quartiniana* leaves had $1c_{50}$ 0.11 ± 0.2µg/ml in brain and 2.97±2.2µg/ml in liver. $1C_{50}$ is the concentration of the extract that caused 50% inhibition of lipid peroxidation.

In order to provide an explanation for the inhibition of Iron (Fe^{2+}) induced peroxidation in rat's brain and liver homogenates the iron chelating ability of the ethanolic extract of *Swertia quartiniana* leaves was determined and the results were shown in figure 1. The result revealed the chelating ability of the ethanolic extract of *Swertia quartiniana* on 1mMFe^{2+} in a dose dependent manner. The extract had maximum chelating ability at concentration of 110µg/ml (76%) and minimum chelating at concentration of 11µg/ml (6%). The chelating ability of the ethanolic extract of *Swertia quartiniana* could have accounted for the higher inhibition of Fe^{2+} - induced lipid peroxidation in rat's brain and liver homogenate. This is because iron plays a catalytic role in the initiation of free radical reaction. The protective ability exhibited by this plant may be due to the presence of antioxidant like Phenols and ascorbic acid whose content was not determined in this study [14, 16].

The resulting oxyradical have the potential to damage cellular lipids nucleic carbohydrate and protein [3]. The overproduction of reactive oxygen specie can directly attack the polysaturated fatty acid of the cell membrane and induce lipid peroxidation [17].

Ethanol, the solvent used as vehicle had no inhibitory effect on lipid peroxidation in the tissues studied. This study indicate that in addition to the inhibitory effect on thiobarbituric acid reactive specie (TBARS), iron chelating ability could be a major contributory mechanism by which *Swertia quartiniana* prevent lipid peroxidation. It was therefore concluded that ethanolic extract of *Swertia quartiniana* prevents iron (Fe^{2+}) induced lipid peroxidation in rat's brain and liver *invitro*. The protective ability is due to its higher phenol content and Fe^{2+} chelating ability.

TABLE 1:The inhibitory effect of ethanolic extract of *Swertia quarhniana* on Iron II sulphate induced lipid peroxidation in rat liver homogenate

Concentration (µg/ml)	MDA(nmole/ gtissue)	% inhibition	Logarithm equation (r^2)	$1c_{50}$ (µg/ml)
Normal	66.7 ± 11.4		y = -37.53	
Control	194.4 ± 11.3		In(x) + 45.67	2.97±2.2
Solvent	185.3 ± 14.9	5.4 ± 20.6	(r^2 = 0.16)	
3.7	139.6 ± 8.7	30.7 ± 7.23		
7.33	249.6 ± 9.7	-6.7 ± 60.0		
14.7	130.2 ±17.8	39.3 ± 15.3		
29	45.6 ± 10.9	69.3 ± 18.5		
37	181.9 ± 8.5	-2.67 ± 23.5		
44	296.9 ± 7.6	-11.1 ± 15.5		
367	382 ± 8.6	-19.7 ± 53.41		

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

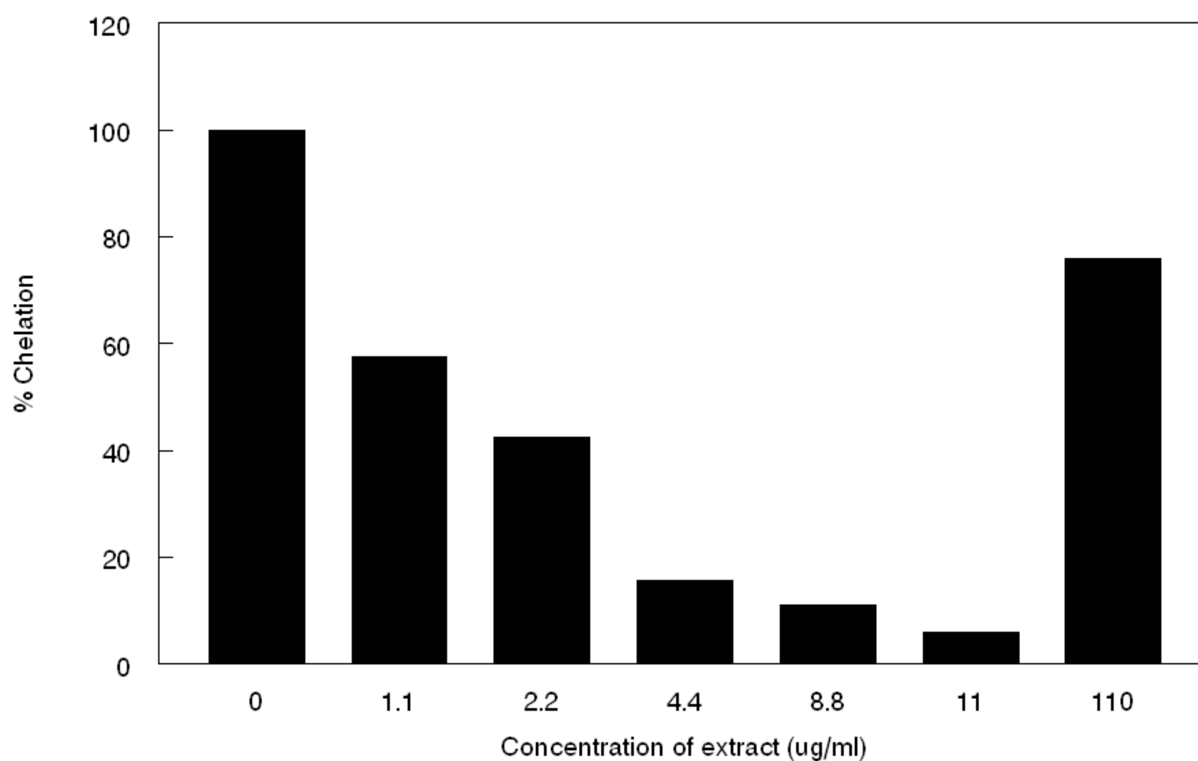

Fig 1 Iron Chelating ability of Ethanolic Extract of *Swertia quartiniana* leaves

TABLE 2:The inhibitory effect of ethanolic extract of *Swertia quartiniana* on Iron II sulphate induced lipid peroxidation in rat's brain homogenate

Concentration (μg/ml)	MDA(nmole/ gtissue)	% inhibition	Logarithm equation (r^2)	1c$_{50}$ (μg/ml)
Normal	79.3 ± 11.2		y = -2.197	0.11 ± 0.2
Control	420.7 ± 5.5		In(x) + 64.69	
Solvent	360 ± 11	-85.9 ± 71.8	(r^2 = 0.0046)	
3.7	205 ± 5.5	48.0 ± 35.7		
7.33	61.5 ± 16.8	82.7 ± 9.2		
14.7	54.47 ± 5.1	72.0 ± 35.5		
29	185.8 ± 15.2	76.0 ± 34.6		
37	209.19 ± 16.7	25.8 ± 23.2		
44	70.7 ± 17.7	84.3 ± 17.6		
367	188.6 ± 12.7	45.3 ± 74.0		

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

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