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Antioxidative Properties of Ethyl Acetate Fraction of *Globimetula braunii* in Normal Albino Rats

¹J. Okpuzor, ²H. Ogbunugafor and ¹G.K. Kareem

¹Department of Cell Biology and Genetics, University of Lagos, Akoka-Yaba, Lagos, Nigeria

²Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

Abstract: *Globimetula braunii* used in Nigerian traditional medicine for managing various diseases was investigated for its antioxidant properties in adult Swiss albino rats. The pulverized plant material was extracted in 80% methanol using Soxhlet apparatus and fractionated with hexane, chloroform, ethyl acetate, butanol and water. The crude and ethyl acetate fractions were evaluated for their effects on activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), lipid peroxidation and triacylglycerol levels in rats treated orally with a daily dose of 200 mg kg⁻¹ for 14 days. Results showed that the ethyl acetate fraction caused a significant increase (p<0.05) in the activities of superoxide dismutase, catalase, glutathione peroxidase and malonyldialdehyde (MDA) levels, while the level of triacylglycerol decreased (p<0.05) compared to control. HPLC analysis showed that the crude and ethyl acetate fractions showed phenolic compounds, apigenin and naringin but only naringin was present in the ethyl acetate fraction. It is likely that endogenous Naringin may be acting *in vivo* both as an antioxidant and a pro-oxidant in normal albino rats.

Key words: Reactive oxygen species, antioxidant enzymes, HPLC, Naringin and Apigenin

INTRODUCTION

Globimetula braunii (Loranthaceae) commonly called Afomo onishano in South West Nigeria is used for the treatment of headache, chest, leg pain and pulmonary problems (Burkill, 1985). It is a bushy, parasitic plant found on a variety of host plants from Ghana to Nigeria and widely dispersed across central tropical Africa (Burkill, 1985). In a developing country like Nigeria where conventional health care is not given adequate attention, a great number of rural dwellers depend solely on traditional health practitioners and medicinal plants. *G. braunii* is one of the plants used by traditional healers in Nigeria to treat various diseases such as cardiovascular diseases, diabetes and sickle cell anaemia.

Recent reports have implicated Reactive Oxygen Species (ROS) as the pathogenesis of many human diseases (Repetto and Llesuy, 2002). Reactive Oxygen Species (ROS) are free radicals generated as by products of normal aerobic metabolism and also from reactions with drugs and toxins (Singh *et al.*, 2005). Excessive production of reactive oxygen species however, results in alteration in the balance between ROS and endogenous antioxidants and creates oxidative stress which is implicated in many pathological conditions such as ischemia/reperfusion injury, diabetes (Choi and Hwang, 2005), cardiovascular disease

(Singh *et al.*, 2005), cancer, Alzheimer's disease and ageing (Surh and Fergusson, 2003), atherosclerosis (Landmesser and Drexler, 2002; Shah *et al.*, 2001) and rheumatoid arthritis (Darlington and Stone, 2001). There is increasing evidence that antioxidants from medicinal plants might have preventive/therapeutic effect on most of these diseases that have aetiology in oxidative stress (Yesilada and Kupeli, 2002; Choi and Hwang, 2005).

Some of these medicinal plants used in ethno medicine for the treatment and management of many of these diseases have been investigated for their antioxidant properties (Aruoma, 2003; Semiz and Sen, 2007). Many of the metabolites from these medicinal plants especially flavonoids exhibited potent antioxidant activity *in vitro* and *in vivo* (Usoh *et al.*, 2005; Sofidiya *et al.*, 2006; Nwanjo, 2007).

Thus, the objective of this study is to investigate the *in vivo* effect of *G. braunii* on antioxidative indices (superoxide dismutase, catalase, glutathione peroxidase, lipid peroxidation) and triglyceride levels to corroborate its use in ethno medicine.

MATERIALS AND METHODS

Plant material: The leaves of *Globimetula braunii* were collected in March 2008, identified and authenticated at the Forestry Research Institute of Nigeria (FRIN) Ibadan,

Nigeria. Voucher specimen was deposited at the institute's herbarium with number FHI 107441.

Animals: Adult Swiss albino rats (mixed sex) were obtained from the animal colony of the Department of Biochemistry, University of Ibadan, Ibadan, Oyo State, Nigeria. Animal use approval was obtained from the University of Lagos Ethical Committee on the use of animals for experimental purposes.

The rats weighing 150-200 g were acclimatized for 7 days and divided into 7 groups of 5 rats each. One group served as control while the rest 6 were the experimental group and they were maintained under standard laboratory conditions while feed and water was given *ad libitum* through out the period of study.

Chemicals and solvent: All chemicals and solvents used for the study were of analytical grade and were obtained from Sigma-Aldrich, Germany.

Extraction of plant material: Three hundred and three grams of pulverized dried leaves of *Globimetula braunii* was extracted with 500 mL of 80% methanol (MeOH) using Soxhlet apparatus. This procedure was repeated twice using 100 mL of the same solvent to ensure that all the extractable components were obtained. The combined MeOH fractions were evaporated to dryness under reduced pressure at below 40°C to yield (35.88 g) of crude drug. The drug/extract yield ratio was 1:8.

Fractionation of plant extract: The method of Yesilada and Kupeli (2002) was adopted with some modifications. The crude methanolic extract of *G. braunii* was reconstituted with 200 mL of MeOH: H₂O (9: 1) mixture and shaken with n-hexane (3×100 mL). Combined hexane extract was evaporated under reduced pressure to yield Hexane fraction (4.95 g). MeOH was evaporated from the remaining extract and diluted with distilled water to 200 mL and further fractionated by successive solvent extraction with chloroform (4×100 mL), ethyl acetate (2×100 mL) and n-butanol saturated with water (3×100 mL). Each extract was evaporated to dryness under reduced pressure to yield chloroform fraction (4.29 g), ethyl acetate fraction (2.87 g), butanol fraction (1.59 g) and water fraction (3.86 g). After preliminary laboratory studies with all the fractions, further experiments were carried out with crude and ethyl acetate fractions only.

High Performance Liquid Chromatography (HPLC) analysis of fraction: HPLC analysis of crude and ethyl acetate fractions were carried out at Department of Clinical and Laboratory Sciences of the University of Lagos Teaching Hospital (LUTH) Lagos, on 1100 series Agilent

Technologies. The automated HPLC system was driven by a CHEM STATION software and the chromatographic separations were performed using a Hypersil 100-5 (Macherey-Nagel) silica gel coated column (250×4.6 mm). The mobile phase selected for the method validation and for the determination of the phenolic compounds were methanol (MeOH), acetonitrile (ACN) and ammonium NH₄ acetate buffer (5:11:4). Before use, the mobile phase was degassed for 15 min in an ultrasonic bath. The samples were monitored with UV detection at 260 nm at the flow rate of 1 mL min⁻¹ at ambient temperature.

Animal treatment: The crude and the ethyl acetate fractions were dissolved in 1% Tween 80 which served as the dosing vehicle. The rats were fed orally for 14 days with 200 mg kg⁻¹ dose of crude and ethyl acetate fractions. Dosage was determined based on preliminary studies in our laboratory.

Enzyme studies

Catalase activity: The method described by Goyal *et al.* (1986) was used. The reaction mixture (1.5 mL) contained 1.0 mL of 0.01 M phosphate buffer pH 7.0, 0.1 mL of serum and 0.4 mL of 2 M H₂O₂. Catalase (CAT) activity was assayed calorimetrically at 620 nm and expressed as specific enzyme activity (mmol min⁻¹).

Superoxide dismutase (SOD) activity: The method of Sun and Zigma, (1978) was adopted. The reaction was carried out by adding (0.02 mL) of serum to (3.0 mL) of 0.05 M sodium carbonate buffer, pH 10.2 and (0.03 mL) of epinephrine in (0.005 N HCl) was used to initiate the reaction. Enzyme activity was calculated by measuring the change in absorbance at 480nm for 5 min. The enzyme activity was expressed as specific enzyme activity (mmol min⁻¹).

Glutathione peroxidase (GSH-Px) activity: Glutathione peroxidase activity was assayed as described by Tappel (1978).

Determination of lipid peroxidation index: The formation of TBARS (thiobarbituric acid reactive substances) was used as an index of lipid peroxidation as described by Niehaus and Samuelson (1968).

Determination of serum triacylglycerol: This was carried out according to the method of Koditschek and Umbreit (1969), using diagnostic kit from Human Gesellschaft für Biochemica und diagnostica, Germany.

Statistical analysis: Results were reported as Mean±SEM of 3 measurements. Test significance was by student's t-test.

RESULTS AND DISCUSSION

The results from this study show significant increase ($p < 0.5$) in the activities of serum SOD, CAT and GSH-Px after the administration of 200 mg kg^{-1} of *G. braunii* ethyl acetate fraction, when compared to the control group (Fig. 1-3). There was also, a significant lowering ($p < 0.5$) of triacylglycerol level ($93.70 \pm 3.31 \text{ mmol L}^{-1}$) in rats treated with ethyl acetate fraction compared to the control group ($129.1 \pm 0.36 \text{ mmol L}^{-1}$) (Fig. 4).

Malonyldialdehyde (MDA) level in the rats treated with ethyl acetate fraction ($20.8 \pm 0.52 \text{ mmol mL}^{-1}$) was significantly increased ($p < 0.05$), compared to control group ($18.2 \pm 0.22 \text{ mmol mL}^{-1}$) (Fig. 5).

HPLC analysis revealed that the crude extract contained both naringin and apigenin (Fig. 6) but only naringin was present in the ethyl acetate fraction (Fig. 7).

The involvement of free radicals in the pathogenesis of certain diseases has been reported and studies have shown that medicinal plants with antioxidative properties

might act as free radical scavengers, reducing agents, chelating agents for transition metals, quenchers of singlet oxygen molecules and activators of antioxidative defence enzymes to suppress free radical damage in

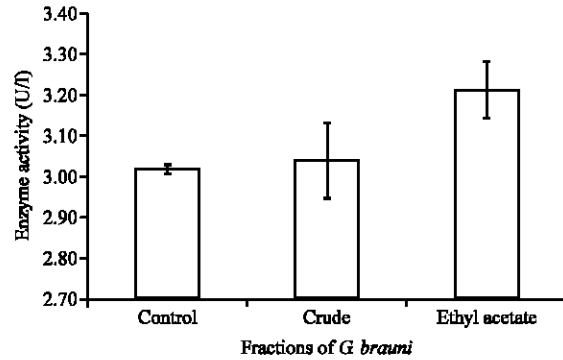


Fig. 3: Effect of crude and ethyl acetate fractions of *G. braunii* on glutathione peroxidase (GSH-Px) activity (U/I) in serum of rats

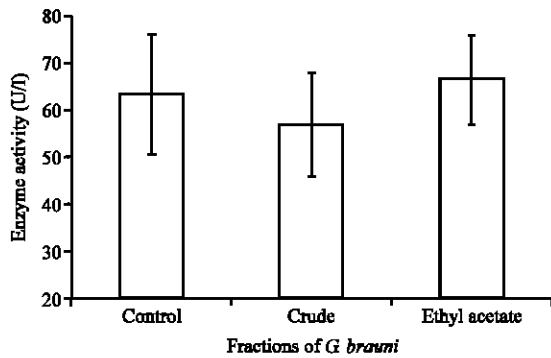


Fig. 1: Effect of crude and ethyl acetate fractions of *G. braunii* on superoxide dismutase (SOD) activity (U/I) in serum of rats

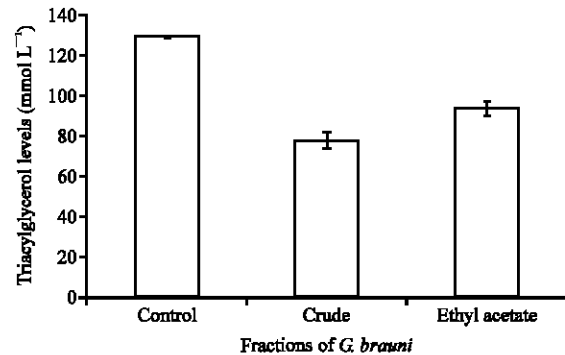


Fig. 4: Effect of crude and ethyl acetate fractions of *G. braunii* on triacylglycerol levels (mmol L^{-1}) in rats

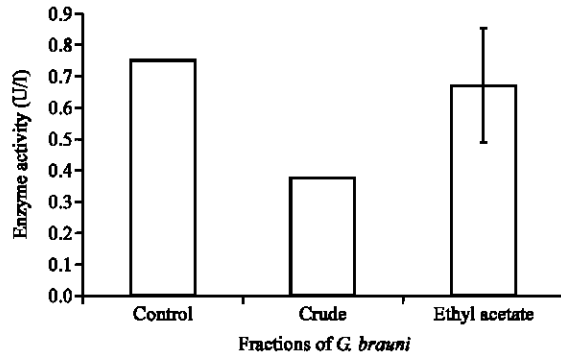


Fig. 2: Effect of crude and ethyl acetate fractions of *G. braunii* on catalase (CAT) activity (U/I) in serum of rats

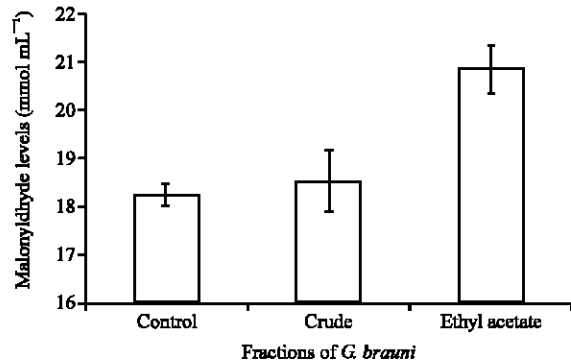


Fig. 5: Effect of crude and ethyl acetate fractions of *Globimetula braunii* on malonyldialdehyde (MDA) levels in normal albino (mmol mL^{-1}) rats

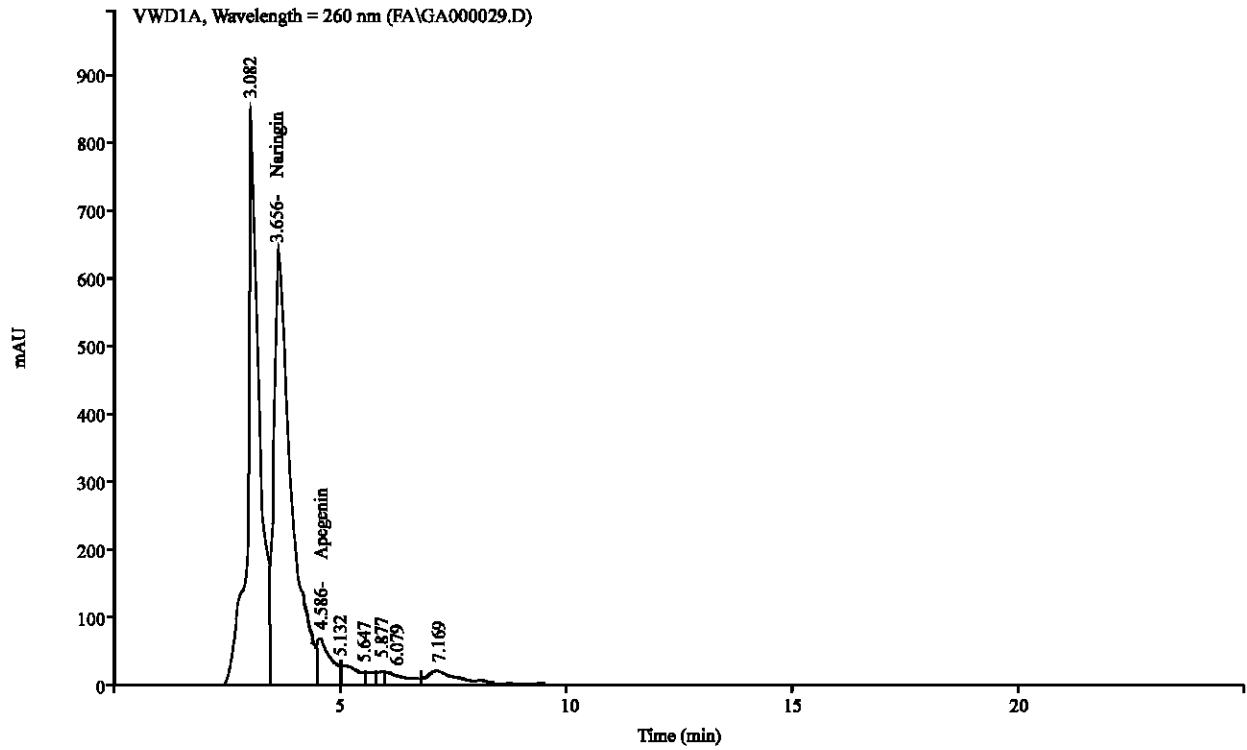


Fig. 6: Elution profile of crude fraction of *Globimetula braunii*

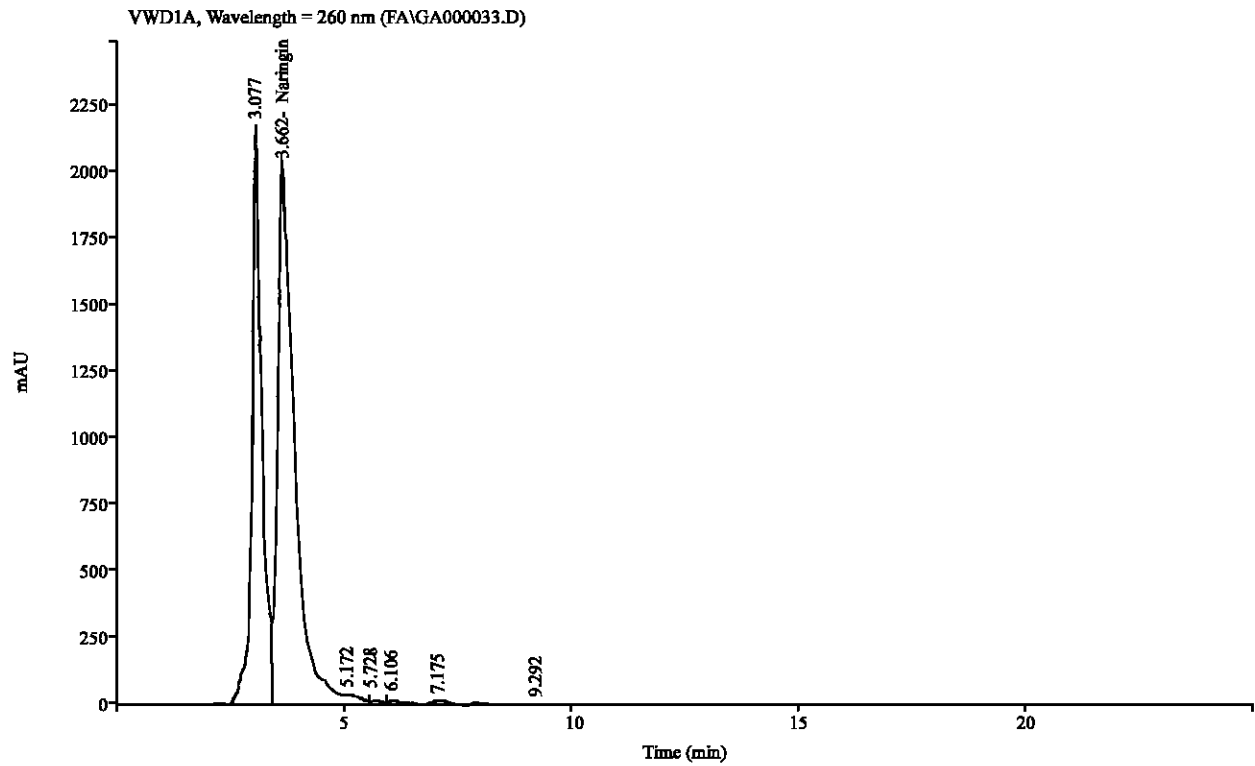


Fig. 7: Elution profile of ethyl acetate fraction of *Globimetula braunii*

biological systems (Finkel and Holbrook, 2000; Sofidiya *et al.*, 2006; Choi and Hwang, 2005).

It is known that phenolic compounds especially flavonoids in plants, are extractable with ethyl acetate and these compounds have been shown to possess antioxidative properties (Wu *et al.*, 2005). The elevated levels of antioxidant enzymes (SOD, CAT and GSH-Px) observed in the ethyl acetate fraction-treated rats may suggest that the components of this fraction might be activating or inducing the synthesis of the enzymes (Finkel and Holbrook, 2000).

This finding is consistent with earlier studies that SOD, CAT and GSH-Px activities and their mRNA levels increased after administration of four different phenolic acids to rats (Yeh and Yen, 2006). This is further supported by earlier studies that have demonstrated the selective induction of manganese superoxide dismutase (MnSOD) or catalase mRNA after exposure of tracheobronchial epithelial cells *in vitro* to different oxidant stresses (Shull *et al.*, 1991). Superoxide dismutase (SOD) has an antitoxic effect against the superoxide anion (O_2^-) and accelerates dismutation of superoxide radicals to hydrogen peroxide (H_2O_2) which is subsequently removed by catalase (Zelko *et al.*, 2002). However, it has been proposed that glutathione peroxidase is responsible for the detoxification of H_2O_2 in low concentration, whereas catalase comes into play when glutathione peroxidase is saturated with substrate (Gaetani *et al.*, 1989).

These studies suggest that over expression of antioxidant enzymes could constitute a powerful chemopreventive approach against adverse effects induced by oxidant pollutants (Wan and Diaz-Sanchez, 2007). Furthermore, some scientists have hypothesized that extra-cellular SOD inhibit pulmonary inflammation by preventing superoxide mediated fragmentation of hyaluronan - a polyanionic polysaccharide found in extra-cellular matrix (Gao *et al.*, 2008).

Triacylglycerols are lipid substances formed from dietary lipids. There is abundant evidence that link heart diseases to high triacylglycerols which result from free radical oxidation of LDL that causes tissue damage (Osawa, 1999). The ability of ethyl acetate fraction to lower triacylglycerol levels *in vivo* buttresses its claimed medicinal benefits especially in the management of cardiovascular diseases.

Naringin, a non-prenylated flavonone, found in citrus fruits such as grape and grapefruit has been shown to be a strong antioxidant which possesses anti-inflammatory activity (Pereira *et al.*, 2007).

Naringin in some studies however, was observed to be a pro-oxidant that promotes rather than limit Low Density Lipoprotein (LDL) oxidation (Buhler and Miranda, 2000). This is supported by present finding of

an increased MDA levels. Silva *et al.* (2002) had also observed that lipid peroxidation was not inhibited when naringin and naringenin were used up to 100 μ M concentrations in their study of structure-antioxidant activity relationships of some flavonoids. This observation, therefore, is consistent with studies that revealed that some of the medicinal plants used in treatment of inflammation such as *M. ciliata*, acts as a prooxidant *in vivo* (Dongmo *et al.*, 2003; Ogbunugafor *et al.*, 2007). This suggests that the antioxidative action of ethyl acetate fraction of *G. braunii* is probably by generation of free radicals *in vivo* which in turn induces antioxidant enzymes.

The findings from this study indicate that naringin which was identified in ethyl acetate fraction of *Globimetula braunii* may be the active component responsible for inducing antioxidative enzymes as well as increased lipid peroxidation in normal albino rats *in vivo*. The lipid lowering effect of the fraction may also be due to this plant metabolite. It is therefore suggested that *Globimetula braunii* possess antioxidant properties. Furthermore, naringin found in the ethyl acetate fraction of *G. braunii* may be the basis for the antioxidative properties of the plant. It is therefore suggested that the plant may be useful in the management of diseases such as diabetes, sickle cell anaemia and cardiovascular diseases where free radicals are often generated.

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