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### Lipid Lowering Activity of *Globimetula braunii*

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**Abstract:** Extract of *Globimetula braunii* in different solvent systems were evaluated for possible lipid and blood pressure lowering activities using *in vivo* and *in vitro* experimental methods. Dried *Globimetula braunii* leaves were pulverized into powder and successively extracted with methanol, hexane, chloroform, ethyl acetate, n-butanol and water using hot extraction methods. Normal male adult albino rats were administered a dosage of 200 mg kg<sup>-1</sup> b.wt. of the extracts for a period of 14 days and the level of total cholesterol, triacylglycerol and lipid peroxidation were monitored. The crude extract of *Globimetula braunii* was analyzed for some antihypertensive substances using High Performance Liquid Chromatography (HPLC). The results obtained, showed that different fractions of the extract caused significant ( $p < 0.05$ ) decrease in serum total cholesterol, triacylglycerol and malonyldialdehyde (MDA) levels. HPLC elution profile showed that the crude extract contained substances similar to some known antihypertensive drugs like propranolol, lisinopril, moduretic and nifedipine and the lisinopril-like compound seems to be the most abundant by having the highest concentration. Thus, the data from this study suggests that *Globimetula braunii* extract contains some biologically active substances that may lower blood pressure and serum lipids.

**Key words:** Triacylglycerol, cholesterol, malonyldialdehyde, antihypertensive drugs, high performance liquid chromatography

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### INTRODUCTION

*Globimetula braunii*, which belongs to the family of Loranthaceae is a parasitic shrub that grows on dicotyledonous trees and attaches itself to the host by modified roots (Burkill, 1985). The members of the Loranthaceae family consist of about seventy-four genera commonly known as mistletoes and are widely distributed in tropical countries like Malaysia and India. They are represented in Cameroon by 26 species grouped into seven genus. Although regarded as a threat to agricultural yield because of its parasitic characteristics (Dibong *et al.*, 2008). Oboh and Nworgu (2008) reports that it is an important medicinal plant which explains the use of the leaves of *Globimetula braunii* to hasten delivery in traditional medicine practice. According to local herb sellers of the Yoruba tribe residing in South western Nigeria, *Globimetula braunii* locally called Afomo onishano is reputed to be effective for treating many diseases ranging from headache, leg pain to pulmonary troubles. There are claims that the leaves, fruits and flowers of the subject plant have been implicated in the management of high blood pressure, while the roots attaching it to the host plant are used for other therapeutic applications like ulcer and cancer treatment (Burkill, 1985). Hypertension is one of the leading causes of death and disability due to complications such as coronary heart disease, stroke, end-stage renal disease and peripheral vascular disease (Khosh and Khosh, 2001).

This investigation seeks to report that *G. braunii* may have blood pressure and lipid lowering activities by depressing the levels of total cholesterol, triacylglycerol and malonyldialdehyde in the serum of albino rats and contains bioactive substances similar to some known antihypertensive drugs.

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## MATERIALS AND METHODS

### Plant Material

The leaves of *Globimetula braunii* were collected in Feb., 2007, from the Forestry Reserve Institute of Nigeria (FRIN) Ibadan, Oyo State, Nigeria. They were identified and authenticated by Mr. Francis Akpan of the Forestry Research Institute of Nigeria (FRIN) and the Herbarium deposit number is FHI 107741.

### Preparation of Crude Extract

The dried leaves of *Globimetula braunii* were pulverized with a blender into a fluffy mass. Three hundred and three grams of powdered leaves of *Globimetula braunii* was extracted with 500 mL of 80% methanol (MeOH) using Soxhlet apparatus. This extraction was repeated three times to ensure that all the extractable components have been removed from the plant material. The extracts were pooled together and this methanol extract served as the crude fraction or extract. The crude extract was evaporated to dryness under reduced pressure and freeze dried to yield 35.88 g of crude extract.

### Fractionation of Crude Extract

Fractionation was done according to the method of Yesilada and Kupeli (2002). The freeze-dried crude methanolic extract of *Globimetula braunii* was then reconstituted with 200 mL of MeOH : H<sub>2</sub>O (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). The methanol fraction was evaporated from the remaining extract and diluted with distilled water to 200 mL and further fractionated by extraction with chloroform (4×00 mL), ethyl acetate (2×100 mL) and n- butanol saturated with H<sub>2</sub>O (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 remaining water fraction (3.86 g).

### Experimental Animals

Normal albino rats were obtained from the animal colony of the Department of Biochemistry, University of Ibadan, Nigeria. The Ethical committee of the University of Lagos approved the use of these animals for experimental purposes. The mature albino rats were allowed to acclimatize for one week in the animal house of University of Lagos before use. The rats weighing between 150-200 g were distributed according to their weights into seven groups of five rats each. The albino rats were kept under standard laboratory conditions and were allowed feed of commercial rat pellets and water *ad libitum* throughout the period of the study.

### Standard Drugs Used for HPLC Studies

The following antihypertensive drugs, Moduretic (Merck sharp and Dohme) Pakistan, Lisinopril (Juhel) Nigeria, Nifedipine (Dexce) Israel and Propanalol (Lek Pharmaceutical) Slovenia, were purchased from University of Lagos Community Pharmacy, Akoka, Yaba, Lagos.

### Acute Toxicity Test

Twenty five albino Wistar mice weighing 17-20 g were used for toxicity studies. The mice were arranged in five groups and each group received one of the following doses 2.5, 5, 10, 15 and 20 mg kg<sup>-1</sup> body weight of the extract orally. The mortality in each cage was assessed 24 h after the administration of the extracts (Miller and Tainter, 1944).

### Treatment with Extracts

The normal albino rats were distributed into seven groups of five rats and each received 200 mg kg<sup>-1</sup> of the extract. The rats in each group were treated with the extracts of the crude, hexane,

chloroform, ethyl acetate, butanol and water fractions of *Globimetula braunii*, respectively except the control group. Each rat received a maximum dose of 1 mL of the extract through the fourteen days period of the study.

#### Collection of Blood Samples

Blood samples were collected from the animals 24 h after the last treatment and further centrifuged at 3000 rpm for 10 min. The plasma decanted was analyzed to evaluate some biochemical parameters.

#### Determination of Cholesterol

Cholesterol was determined after enzymatic hydrolysis and oxidation. This was as described by the protocol provided by the kit manufacturer Human Gesellschaft Fur Biochemica Und Diagnostica, Germany.

One milliliter of working reagent (Phosphate buffer pH 6.5 (100 mmol L<sup>-1</sup>), 4-aminophenazone (0.3 mmol L<sup>-1</sup>), phenol (5 mol L<sup>-1</sup>), peroxidase (>5 KU L<sup>-1</sup>), cholesterolesterase (>150 U L<sup>-1</sup>), cholesteroxidase (>100U L<sup>-1</sup>, 0.05% sodium azide) was added to both 0.01 mL<sup>-1</sup> of serum and standard (5.17 mmol L<sup>-1</sup> cholesterol) in separate test tubes. A reagent blank was then prepared containing 1 mL of the working reagent. The solutions were mixed appropriately and incubated for 10 min at 25°C. The absorbance of serum and the standard was measured against the reagent blank at 500 nm at 30 min.

Cholesterol concentration was calculated according to the following formula:

$$C = 200 \times \frac{\Delta A(\text{Sample})}{\Delta A(\text{Standard})} (\text{mg dL}^{-1})$$

#### Lipid Peroxidation Indices (TBARS)

The formation of TBARS (Thiobarbituric Acid Reactive Substances) was used as an index of lipid peroxidation as described by Niehaus and Samuelson (1968). 0.1 mL of serum was treated with 2 mL of thiobarbituric acid (0.37%), trichloroacetic acid (15%) and 0.25 M HCL all in ratio 1:1:1. The reaction mixture was placed in boiling water bath for 15 min, cooled and centrifuged at room temperature for 10 min at 1000 rpm. The absorbance of clear supernatant was measured against reference blank at 535 nm.

#### Triacylglycerol Concentration

The triacylglycerols were determined after enzymatic hydrolysis with lipases. This was carried out according to the protocol provided by the kit manufacturer (Human Gesellschaft Fur Biochemica Und Diagnostica, Germany). One milliliter of the working reagent {(4-chlorophenol (5 mmol L<sup>-1</sup>), PIPES buffer pH 7.5 (50 mmol L<sup>-1</sup>), 4-aminoantipyrine (0.25 mmol L<sup>-1</sup>), magnesium ions (4.5 mmol L<sup>-1</sup>), ATP (2 mmol L<sup>-1</sup>), lipases (1.3 U mL<sup>-1</sup>), peroxidase, POD (0.5 U mL<sup>-1</sup>), Glycerol kinase, GK (0.4 U mL<sup>-1</sup>), Glycerol-3-Phosphate oxidase, GPO (1.5 U mL<sup>-1</sup>)} was added to 0.01 mL<sup>-1</sup> of blood sample and to 3 mL of standard (2.28 mmol L<sup>-1</sup> of triacylglycerol). A reagent blank was also prepared containing only 1 mL<sup>-1</sup> of working reagent. The solutions were mixed appropriately and then incubated for 10 min at 25°C. Thereafter, the absorbance of the standard ( $\Delta A_{\text{std}}$ ) and serum ( $\Delta A_{\text{sample}}$ ) were measured against the reagent blank at 60 min.

Calculation of triacylglycerol concentration is given as:

$$C = 200 \times \frac{\Delta A(\text{Sample})}{\Delta A(\text{Standard})} (\text{mg dL}^{-1})$$

### Statistical Analysis of Data

Data were expressed as Mean Standard Error (Mean±SEM). Statistical differences between the treatments and the control were tested by two tailed Student's t-test.  $p < 0.05$  was considered significant.

### High Performance Liquid Chromatography (HPLC)

HPLC was carried out using HPLC 1100 series from Agilent technologies. The chromatographic conditions consist of column- Zorbax eclipse XDB, RP (150×4.6) mm and particle size of 5  $\mu\text{m}$ , mobile phase methanol: 25 Mm sodium phosphate buffer (40: 60)%, pH 3.01. Temperature was set at 30°C, flow rate of 1 mL  $\text{min}^{-1}$  and the wavelength VWD detector was set at 254 nm. Twenty micro litres of *Globimetula braunii* crude extract as well as standard antihypertensive drugs moduretic, Lisinopril, Propanolol and Nifedipine were injected and analyzed automatically by Enhanced integration. The antihypertensive drugs served as reference samples.

## RESULTS AND DISCUSSION

So many research findings have proven that lowering the plasma lipids could minimize the complications associated with atherosclerosis and cardiovascular events (Ghasi *et al.*, 2000; Baradaran and Nasri, 2006).

The result of acute toxicity studies in rats revealed that *Globimetula braunii* has no toxic effects at the various doses used for this study. Drugs derived from plant origin are considered less toxic and exhibit fewer side effects compared to synthetic ones (Pari and Umamaheswari, 2000). It was observed that while all the fractions had cholesterol lowering activity, the crude fraction had the greatest significant reduction of cholesterol level by about 50% whereas ethyl acetate fraction indicated otherwise (Table 1). Several studies have been undertaken to determine the effective means of reducing hyperlipidemia and researchers have beamed their search-light mostly on natural products. Thus, it has been suggested that garlic and preparations derived from it, is an effective hypolipidemic agent. A decrease in serum cholesterol, serum triacylglycerol and a significant decrease in LDL - cholesterol in hyperlipidemics were observed in patients treated with garlic confirming its efficacy (Ziaei *et al.*, 2001; Rahman and Billington, 2000; Superko and Krauss, 2000; Byrne *et al.*, 1999). It has also been reported by Sharma *et al.* (2007) that leaf extract of *Aegle marmelos* significantly reduced serum total cholesterol and triacylglycerol in diabetic mice. They suggested that the cholesterol lowering activity might be due to the constituents of the plant extract that may act as inhibitors of some enzymes such as hydroxyl-methyl-glutaryl-CoA reductase, which participates in *de novo* biosynthesis of cholesterol.

There was a significant reduction ( $p < 0.05$ ) in triacylglycerol levels in all the fractions compared to the control group, with the butanol fraction showing the highest reduction in triacylglycerol levels (Table 2). High levels of triacylglycerols in the bloodstream have been linked to atherosclerosis and predispose an individual to the risk of stroke and heart disease (Ghasi *et al.*, 2000). Banerjee and Maulik (2002) stated that the major etiopathological index for atherosclerosis is hyperlipidemia and herbs and dietary supplements can have hypolipidemic effects as reported by Kris-Etherton *et al.* (2002). Crataegus species could be beneficial to patients with cardiovascular diseases as shown from some studies (Fong and Bauman, 2002; Fugh-Berman, 2000).

Lipid peroxidation studies showed that there was significant decrease ( $p < 0.05$ ) in malonyldialdehyde levels of chloroform and hexane fractions of *Globimetula braunii* compared to the control group (Table 3). However, the malonyldialdehyde levels of the crude extract compared favourably with control. Malonyldialdehyde (MDA) results from the peroxidation of biological membrane polyunsaturated fatty acid. MDA is used as an indicator of tissue damage involving a series

Table 1: Effect of different fractions of *Globimetula braunii* on cholesterol levels in normal albino rats

| Fractions     | Mean±SEM      | p-value (control) |
|---------------|---------------|-------------------|
| Control       | 162.29±5.39   | 0                 |
| Crude         | 84.68±6.13*** | 0.0003            |
| Hexane        | 141.58±3.93*  | 0.0240            |
| Chloroform    | 113.67±6.1    | 0.0017            |
| Ethyl acetate | 186.53±14.67  | 0.1957            |
| Butanol       | 106.74±7.23   | 0.0023            |
| Water         | 155.69±7.51   | 0.5618            |

\* is significant and \*\*\* is most significant; SEM: Standard Error of Mean

Table 2: Effect of different fractions of *Globimetula braunii* on triacylglycerol levels in normal albino rats

| Fractions     | Mean±SEM      | p-value (control) |
|---------------|---------------|-------------------|
| Control       | 129.10±0.36   | 0                 |
| Crude         | 77.88±4.25*** | 0.0002            |
| Hexane        | 86.33±2.95*** | 0.0001            |
| Chloroform    | 80.43±8.49**  | 0.0051            |
| Ethyl acetate | 93.70±3.31*** | 0.0003            |
| Butanol       | 68.90±1.79*** | 0.0001            |
| Water         | 88.15±4.51    | 0.0005            |

\*\* is more significant and \*\*\* is most significant; SEM: Standard Error of Mean

Table 3: Effect of different fractions of *Globimetula braunii* on malonyldialdehyde levels in normal albino rats

| Fractions     | Mean±SEM    | p-value (control) |
|---------------|-------------|-------------------|
| Control       | 18.23±0.22  | 0                 |
| Crude         | 18.51±0.67  | 0.734             |
| Hexane        | 17.33±0.17* | 0.0226            |
| Chloroform    | 17.31±0.14* | 0.0104            |
| Ethyl acetate | 20.78±0.52  | 0.0229            |
| Butanol       | 20.06±1.13  | 0.2311            |
| Water         | 19.33±0.75  | 0.3172            |

\* is significant; SEM: Standard Error of Mean

of chain reactions (Bhutia *et al.*, 2006). It is possible that the crude extract contains substances acting synergistically with some other components that have been completely removed by successive fractionation of the crude in different solvent systems. Lipid peroxidation has been implicated in the pathogenesis of increased membrane rigidity, reduced erythrocyte survival and perturbation in lipid fluidity (Ochoa *et al.*, 2003). Ameer *et al.* (2008), reported that the chloroform fraction of *Loranthus ferrugineus* produced a significant drop in noradrenaline induced aortic rings contraction. Wegwu *et al.* (2005) had also observed that the crude methanolic extract of *Cassia alata* pods conferred hepatoprotective antioxidant capacity on rats treated with carbon tetrachloride, which is known to cause lipid peroxidation owing to high malonyldialdehyde content.

The *in vitro* results from the elution profile HPLC studies revealed that *G. braunii* has bioactive substances similar to some known anti-hypertensive drugs (Table 4). These bioactive substances were observed to contain a mixture of Nifedipine, Propanolol, Lisinopril and Moduretic in different concentrations. The concentrations were calculated from HPLC retention times and Lisinopril had the highest concentration. The observation shows that the bioactive substances identified from this study possess lipid lowering activity. This will help in reducing tension in blood circulation and thereby improving flow. The mechanism of action of the plant extract is yet to be elucidated. However, the HPLC analysis of the extract showed compounds similar to the constituents of standard anti-hypertensive drugs based on the current *in vitro* study. We therefore, suggest that this plant extract may contain some Angiotensin- Converting Enzyme (ACE) inhibitors or beta blockers whose activities may have contributed to the reduction of the indices of cardiovascular diseases.

The conclusion from this study affirms from *in vitro* and *in vivo* studies, that *G. braunii* has great potentials as a lipid lowering agent based on the proven ability of the extract to lower cholesterol,

Table 4: Concentration of bioactive substances similar to standard drugs present in the *G. brauni*

| Standard drugs | Retention times (min) | Concentration of bioactive compounds ( $\mu\text{g mL}^{-1}$ ) |
|----------------|-----------------------|--|
| Lisinopril     | 2.61                  | 1320.83  |
| Moduretic      | 1.75                  | 997.48   |
| Propranolol    | 10.15                 | 719.88   |
| Nifedipine     | 6.00                  | 0.06   |

triacylglycerol and lipid peroxidation in rats. Accordingly, the presence of the mixture of some known anti-hypertensive drugs, lends credence to this suggestion. Finally, intensive human trial is recommended to ensure that the full benefits of using this affordable treatment option is not lost on the rural inhabitants of the developing world where this plant may be readily available.

## REFERENCES

- Ameer, O.Z., I.M. Salman, M.F. Yam, M.H. Abdullah and M.Z. Asmawi, 2008. Vasorelaxant properties of *Loranthus ferrugineus* Roxb. Methanolic extract. Int. J. Pharmacol., 4: 472-476.
- Banerjee, S.K. and S.K. Maulik, 2002. Effect of garlic on cardiovascular disorders: A review. Nutr. J., 1: 1-14.
- Baradaran, A. and H. Nasri, 2006. Serum cholesterol as a marker of nutrition in end-stage renal failure patients on replacement therapy. Int. J. Pharmacol., 2: 184-187.
- Bhutia, R.D., B. Upadhyay and M. Maneesh, 2006. Association of plasma level of thiobarbituric acid reactive substances with extent of hepatocellular injury in preterm infants with cholestatic jaundice. Indian J. Clin. Biochem., 21: 39-41.
- Burkill, H.M., 1985. The useful Plants of West Tropical Africa. Vol. 1, Ithaca Harbors Incorporation, UK., ISBN: 0-947643-64-8, pp: 369.
- Byrne, D.J., H.A. Neil, D.T. Vallance and A.F. Winder, 1999. A pilot study of garlic consumption shows no significant effect on markers of oxidation or sub-fraction composition of low-density lipoprotein including lipoprotein (a) after allowance for non-compliance and the placebo effect. Clin. Chim. Acta., 285: 21-33.
- Dibong, S.D., R.J. Priso, V.D. Taffouo, H. Fankem, G. Salle and A. Amougou, 2008. Parasitism of host trees by Loranthaceae in the region of Douala (Cameroon). Afr. J. Environ. Sci. Toxicol., 2: 371-378.
- Fong, H.H.S. and J.L. Bauman, 2002. Hawthorn alternative medicines for cardiovascular diseases. J. Cardiovascular Nursing, 16: 1-8.
- Fugh-Berman, A., 2000. Herbs and dietary supplements in the prevention and treatment of cardiovascular disease. Prev. Cardiol., 3: 24-32.
- Ghasi, S., E. Nwobodo and J.O. Ofili, 2000. Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed wistar rats. J. Ethnopharm., 69: 21-25.
- Khosh, F. and K. Khosh, 2001. A natural approach to hypertension. Altern. Med., 6: 590-600.
- Kris-Etherton, P.M., K.D. Hecker, A. Bonanome, S.M. Coval and A.E. Binkoski *et al.*, 2002. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. Am. J. Med., 30: 71S-88S.
- Miller, L. and M. Tainter, 1944. Estimation of EC50 and its error by means of log. Probit. Graph. Paper Proc. Soc. Exp. Biol. Med., 26: 1-9.
- Niehaus, Jr. W.G. and B. Samuelson, 1968. Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur. Biochem., 6: 126-130.
- Oboh, I.E. and Z.A.M. Nworgu, 2008. Oxytocic properties of the aqueous extract of *Globimetula braunii* (Loranthaceae). Pak. J. Pharm. Sci., 21: 356-360.

- Ochoa, J.J., M.J. Vilchez, M.A. Palacios, J.J. García, R.J. Reiter and A. Muñoz-Hoyos, 2003. Melatonin protects against lipid peroxidation and membrane rigidity in erythrocytes from patients undergoing cardiopulmonary bypass surgery. *J. Pineal. Res.*, 35: 104-108.
- Pari, L. and J. Umamaheswari, 2000. Antihyperglycemic activity of *Musa sapientum* flowers: Effect on lipid peroxidation on alloxan induced diabetic rats. *Phytoterapy Res.*, 14: 1-13.
- Rahman, K. and D. Billington, 2000. Dietary supplementation with aged garlic extract inhibits ADP-induced platelet aggregation in humans. *J. Nutr.*, 130: 2662-2665.
- Sharma, B., S.K. Satapathi and P. Roy, 2007. Hypoglycemic and hypolipidemic effects of *Aegle marmelos* (L) leaf extract on streptozotocin induced diabetic mice. *Int. J. Pharmacol.*, 3: 444-452.
- Superko, H.R. and R.M. Krauss, 2000. Garlic powder, effect on plasma lipids, postprandial lipemia, low-density lipoprotein particle size, high-density lipoprotein subclass distribution and lipoprotein (a). *J. Am. Coll. Cardiol.*, 35: 321-326.
- Wegwu, M.O., E.O. Ayalogu and J.O. Sule, 2005. Antioxidant protective effects of *Cassia alata* exposed to carbon tetrachloride. *J. Applied Sci. Environ. Mgt.*, 9: 73-80.
- Yesilada, E. and E. Kupeli, 2002. *Berberis crataegina* DC. exhibits potent anti-inflammatory analgesic and febrifuge effects in mice and rats. *J. Ethnopharmacol.*, 79: 237-248.
- Ziaei, S., S. Hantoshzadeh, P. Rezasoltani and M. Lamyian, 2001. The effect of garlic tablet on plasma lipids and platelet aggregation in nulliparous pregnant at high risk of preeclampsia. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 99: 201-206.