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Evaluation of the nitric oxide scavenging potential of *Harungana madagascariensis* Lam ex poir (Hypericaceae) fruits oil

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Abstract
This study investigated the *in vitro* nitric oxide scavenging activity of the oil extract from the non-utilised fruits of *Harungana madagascariensis* a plant widely used in ethno-medicine. The *H. madagascariensis* fruits oil (HMO) was obtained by de-fatting the chloroform extract of the air-dried fruits with n-hexane and the n-hexane extract further separated on a normal phase silica gel column to afford the HMO. Nitric oxide (NO) scavenging assay was done using standard spectrophotometric method. The HMO exhibited a concentration dependent NO scavenging potential comparable (p=0.05) to that of Vitamin A at 50 µg/ml. Compared to the reference antioxidants used for comparison the trend in NO activity inhibition IC_{50} (µg/ml) of : HMO(IC_{50}=214.1)<Vit.A(IC_{50}=146.0)<Vit.C(IC_{50}=128.1)<Vit.E(IC_{50}=96.4) was observed. The observed NO scavenging activity profile of the HMO pointed to the nutraceutical benefits of the oil extract from the fruits of *H. Madagascariensis* thus validating some of the ethno-medicinal uses.

Keywords: *H. Madagascariensis*, non-utilised fruits, oils extract, antioxidant

Introduction
Oxidative stress caused by over production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been associated with the progression of several diseases of ageing/degeneration, inflammation and complications associated with diabetes, anaemia and cardiovascular diseases. The use of exogenous plant antioxidants in form of nutraceutical supplements in the management of diseases whose pathophysiology is linked to oxidative stress is currently of high interest. They have been helpful in the regeneration of damaged tissues due to the harmful effects of free radicals like the ROS and RNS by restoring the balance between their production and neutralization when endogenous antioxidant mechanisms fail to quench the free radicals [1]. *Harungana madagascariensis* is a plant widely used in ethnomedicine. Commonly called dragon's blood tree, the leaves and stem bark are used for the treatment of anaemia, the stem bark is also used for nephrosis, malaria, gastro-intestinal disorders, skin and bacterial infections, and fever [2-10]. It is a component of Jubi Formula, a herbal preparation which was found to restore the pack cell volume and haemoglobins concentration in anaemia conditions [3].The *in vitro* anti-plasmodial effects of six isolated compounds from the root bark of *Harungana madagascariensis* have been reported [11]. Although several pharmacological studies on the roots, stems and leaves of *H. madagascariensis* have been reported [2-11], literature report on the pharmacological activities of the fruits are few [12]. As a follow-up to our earlier report on the fruit of this plant [12] aimed at establishing the health benefit of this non-utilised fruits, and considering the role played by oxidative stress in the pathophysiology of various diseases associated with its reported ethnomedicinal uses, this present study reported the nitric oxide scavenging potential of the fruits oil extract from this plant

Materials
Plant materials
The fruits of *H. madagascariensis* were collected from the forest adjourning the University of Port Harcourt, Nigeria and authenticated at the Herbarium unit of the Plant Science and Biotechnology Department of the same University with Voucher Number: UPH/P/080; UPH/V/1,219

Reagents and Instruments
Reagents and solvents used in this study were of analytical grade and are products of BDH and Sigma-Aldrich.

Methods
Preparation of the extract
The cold maceration technique was for the extraction of the pulverised fruits (500 g). This is to preserve the integrity of thermo-labile constituents that could be present. Chloroform was used as the solvent for extraction. The chloroform extract obtained was then defatted by exhaustive cold maceration in n-hexane to afford the n-hexane extract (crude oil extract) after drying. The crude oil extract was further chromatographed on a normal phase silica gel column with a 5% stepwise gradient of n-hexane-chloroform (100:0 - 90:10 v//v) as mobile phase to afford a highly viscous liquid which was then treated with acetone to precipitate steroidal impurities and from the supernatant steroid free layer was obtained the *H. madagascariensis* fruit oil HMO after evaporating the acetone

Nitric oxide scavenging assay
This was based on the Greiss Illosvoy reaction [13]. The reaction mixture containing 10mM sodium nitroprusside in 0.5 M phosphate buffer, pH 7.4, and the various concentration of the oil (1000, 750, 500, 250, 100, and 50 µg/mL) to a final volume of 3 mL were incubated for 60 minutes at 37°C. Greiss reagent (0.1% aqueous alpha-napthyl-ethylenediamine and 5% sulphanillic acid in 1% aqueous H_3PO_4) was then added to the reaction mixture after incubation. The concentration of the pink chromophore generated due to the diazotization of the nitrite ion with sulphaniliamide and subsequent coupling with the alpha-napthyl-ethylenediamine was measured spectrophotometrically at 540 nm with Vit. A, C and E were used as reference standards for positive control. All these procedures were done in triplicate. The IC_{50} (the concentration that gave 50% inhibition of NO activity) was extrapolated from a plot of absorbance against the concentration through regression analysis.
Percentage nitric oxide activity = 100[(Ao-As)]/Ao
Where Ao= Absorbance of the blank, As = absorbance of the test sample

Statistical analysis
One way ANOVA, student t test of significance at 95% confidence level, and regression analysis were used to analyse the mean of experimental data due to triplicate analysis.

Results and Discussion
The HMO exhibited a concentration dependent NO scavenging (Table 1 and Figure 1) antioxidant potential. At 50 µg/ml, the HMO and the reference standard compound Vitamin A showed comparable NO scavenging potential (p=0.05). However, a significantly lower NO scavenging activity was observed compared to the other reference standard antioxidants vitamin C and E. Generally, a trend in NO activity inhibition IC_{50} (see Figure 2 of HMO <Vit. A< Vit. C< Vit. E was observed. Nitric oxide is a chemical mediator involved in the regulation of several physiological processes. It is generated from the amino acid L- arginine in biological tissues by macrophages, neurons and endothelial cells. Over production of nitric oxide and related reactive nitrogen species are associated with several diseases like cancer, Alzheimer, arthritis multiple sclerosis, complications in diabetes, and ulcerative colitis among others [14-16]. Through its unpaired electrons, NO reacts with proteins thereby causing alteration in the structure and function of many cellular components leading to DNA fragmentation, damage to cells and eventual cell death [17]. The toxic effect of NO becomes adverse when on reacting with superoxide radical, form the highly reactive peroxynitrite anion [18].

Table 1: Nitric Oxide scavenging activity of HMO compared to Vitamins A, C and E

Test concentration (µg/ml)	% nitric oxide scavenging activity of test samples			
	VIT A	VIT C	VIT E	HMO
50	33.76±0.36*	41.10±0.12	40.09±1.53	34.09±0.97*
100	49.57±0.41	50.78±0.27	52.70±0.47	42.40±0.21
250	63.42±0.23	53.89±0.36	66.10±0.18	54.88±0.36
500	65.48±0.18	59.95.0±0.24	78.24±0.27	57.21±1.26
750	76.15±0.15	74.81±0.27	86.27±0.12	68.28±0.09
1000	83.51±0.23	80.32±0.29	92.56±0.18	74.38±0.24
IC_{50}(µg/ml)	146.04	128.08	96.42	214.11

*Not significantly different (p=0.05)

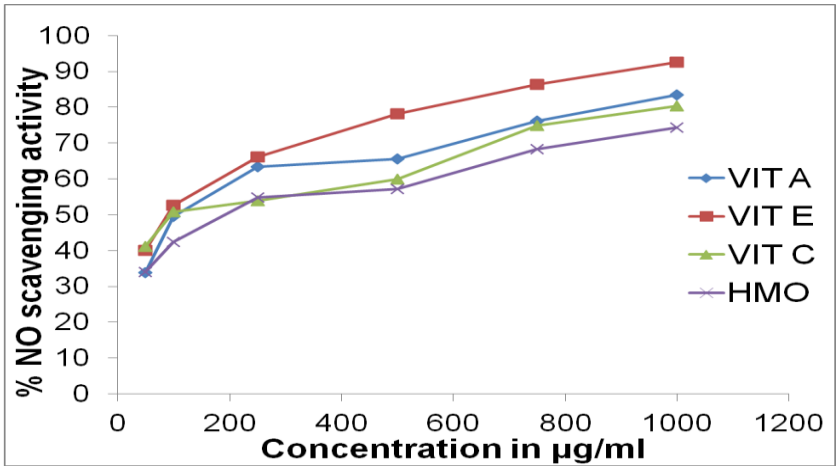

Fig 1: Nitric oxide scavenging activity of HMO compared to Vitamins A, C and E

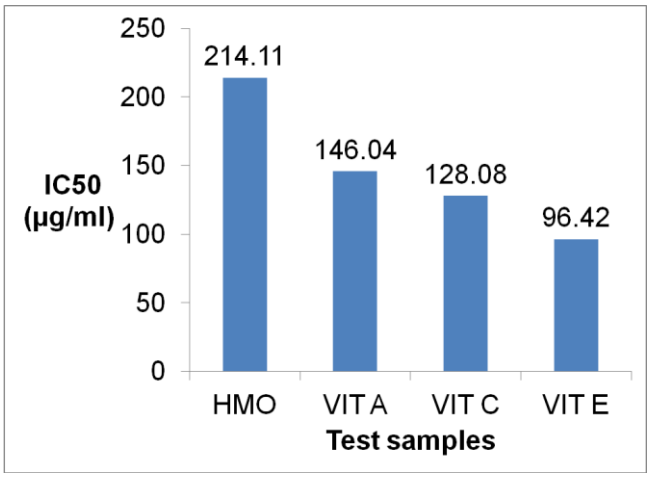

Fig 2: Trend in IC_{50} for Nitric oxide scavenging activity of HMO, Vitamins A, C and E

Conclusion

The fruit oil extract from *H. Madagascariensis* has antioxidant potential as seen from its ability to scavenge for NO radicals. This pointed to its being used as a natural source of antioxidant nutraceutical in management of diseases of degeneration and inflammation associated with oxidative stress.

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