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RESEARCH ARTICLE

IN-VITRO ANTIOXIDANT, LIPID PEROXIDATION INHIBITION AND LIPID PROFILE MODULATORY ACTIVITIES OF HB CLEANSER®BITTERS IN WISTAR RATS

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Abstract

Background: HB cleanser® bitters is a polyherbal formulation with six medicinal plants as phytoconstituents which is being sold to the public for the treatment of various diseases. Hence, it becomes pertinent to evaluate the likelihood of health issues that may be associated with its consumption to provide information to the public on the biological activity and safety.
Objectives: This study was conducted to investigate the *in vitro* antioxidant, lipid peroxidation inhibition and lipid profile effects of HB cleanser® bitters in Wistar rats.
Methods: *In vitro* antioxidant activity was carried using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay, nitric oxide scavenging activity, ferric reducing antioxidant power assay. Inhibitory activity on lipid peroxidation was also measured. Phytochemical evaluation was done. Twenty-eight male rats were allotted into four groups of seven animals each. Group A received 5 ml/kg normal saline while groups B, C and D were administered with 1 ml/kg, 1.03 ml/kg and 1.29 ml/kg of the bitters based on the manufacturer's recommendation through the oral route for 28days consecutively. Lipid parameters assayed were total cholesterol, total triglyceride, high density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C).
Results: Phytochemical screening indicated the presence of flavonoids and saponins. The antioxidant activity of HB cleanser® bitters was dose dependent as it significantly ($p<0.05$) increased with increase in concentration when compared with ascorbic acid. HB bitters®at 1000 µg/ml significantly ($p<0.05$) inhibited lipid peroxidation (78.21±0.53 %) compared to ascorbic acid (94.43±0.53 %) *in-vitro*. The bitters at 1.29 ml/kg exhibited a non-statistically significant ($p>0.05$) decrease of total cholesterol and total triglyceride (2.32±0.15 mmol/L, 0.92±0.13 mmol /L) with a marked increase in low density lipoprotein-cholesterol (1.32±0.20 mmol/L) compared to control.
Conclusion: The findings of this study have revealed that HB cleanser®bitters possesses good antioxidant activity and may increase low- density lipoprotein-cholesterol, therefore it should be used with caution.
Keywords: HB cleanser bitters; *in-vitro*; antioxidant; total cholesterol; triglyceride.

INTRODUCTION

Stem barks, leaves, nuts, and roots of plants have been in use traditionally for a long time for the treatment of diverse ailments with huge success rate even before the introduction of allopathic medicines. However, their use has been surpassed with the discovery and development of synthetic drugs for a long time. There is a re-emergence and recovery in the use of herbal medicines all over the world with many people embracing it even in sub- Saharan Africa[1].

Herbal medicines are openly displayed, advertised, sold, and marketed even on the streets which accounts for the increase in their use, either alone or in combination with allopathic medicines[1]. Herbal bitters are usually poly-herbal in nature and formulation which most often are in liquid form. Other dosage forms include capsules, tablets and tinctures which are usually labelled by their manufacturers as bitters. These bitters may be beneficial in humans but may not be completely harmless[2].

HB cleanser® bitters advertisement says it is being used in the treatment of malaria, waist pain, typhoid, and infections. Its contents include, *Aloe vera, Acino sarvensis, Moringa oleifera, Chenopodiastrum murale, Cinnamomum aromaticum, Allium sativum. Aloe vera* contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids. Aloe vera has been reported to reduce the blood glucose concentration, decrease hepatic transaminases, total cholesterol, triglycerides, phospholipids, and free fatty acids[3], possess antibacterial and antifungal properties[4] and is used in the treatment of digestive system conditions such as constipation, irritable bowel syndrome and ulcerative colitis. *Acinosarvensis* (Lamiaceae) popularly known as basil thyme and spring savoury, is a species of the Acinos genus. The scent is faintly reminiscent of thyme, giving it its common name[5]. Studies have shown that *Acinos arvensis* oil has potent anticancer, antiviral, antimicrobial and powerful antioxidant activities[6].

Moringa oleifera (Moringaceae) with common names such as moringa, drumstick tree has antioxidant[7], antidiabetic[8], cardiovascular[9] and anti-inflammatory activities[10]. *Moringa oleifera*is used as antioxidant due to it bioactive components vitamin and polyphenol[11].

Chenopodia strummurale (Amaranthaceae) is a species of plant in the amaranth family known by the common names nettle-leaved goosefoot, Australian-spinach, salt-green, and sowbane which have been reported to possess antifungal and antibacterial properties[12].

Cinnamomum aromaticum (Lauraceae) is one of several species of Cinnamomum used primarily for their aromatic bark, which is used as a spice. Cinnamomum has been reported to possess hepato-protective[13], antimicrobial[14] and anti-tumor activities[15].

Allium sativum (Amaryllidaceae) widely referred to as garlic is a species in the Allium genus and is closely associated with onion, shallot, leek, chive and Chinese onion[5] which has antimicrobial, anti-aging, anticancer and antifungal biological activities[16] and lowers the risk of cardiovascular disease and diabetes. Herbal remedies have also become a household product in developed countries alongside complementary and alternative medicines in Europe, North America, and Australia[17]. The increase in the sale, use and consumption of herbal medicinal preparations has led to increase in the awareness of public health safety concerns. Quite a number of these products are neither registered nor approved or subjected to standardization protocols by the regulatory authorities which make some class of people sceptical about their safety and efficacy[18]. This is coupled with the fact that some of these preparations lack adequate information on dosage administration, contraindications or likely drug-drug or drug herb interactions on their labels or packages[19].

To this end, it has become appropriate, therefore, to assist the public including healthcare professionals with adequate information to facilitate a better under-standing of the risks that may likely be associated with the use of these products and to ensure that all medicines are safe and of suitable quality. Therefore, this study aimed to provide among others, information to add to the existing literature by investigating the antioxidant activity, lipid peroxidation inhibitory activity and effects on lipid profile (total cholesterol, total triglyceride, high density lipoprotein-cholesterol and low-density lipoprotein-cholesterol) of HB cleanser® bitters.

MATERIALS AND METHODS

Purchase of HB Cleanser Bitters®

HB cleanser® bitters was purchased from Luckystar branch Office, Mile 3, Port Harcourt, Rivers State, Nigeria. HB cleanser® bitters was bought as liquid formulations and stored at room temperature throughout the period of the experiment.

Ethics approval

Ethics approval was obtained from the research ethics committee of the University of Port Harcourt with the approval number UPH/R and D/REC/04 in compliance with international standards.

Animals Used

Twenty-eight male albino rats of the Wistar strain were obtained from the Faculty of Pharmaceutical Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria. They were housed in a well-ventilated room in the animal house of the Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port-Harcourt, Rivers State, Nigeria under standard conditions and were allowed to have access to water and feed *ad libitum.*

Phytochemical Screening

Phytochemical screening was conducted on HB cleanser® bitters to determine the phytochemical constituents of its composition. Tests conducted include tests for alkaloid, free anthraquinone, combined anthraquinone, steroids/triterpenoids, cardiac glycosides, carbohydrate, tannins, phlobatannin, flavonoids and saponins[20].

Test for Alkaloids

To 1mL of the HB cleanser® bitters 5ml of 5%v/v aqueous hydrochloric acid was added. The mixture was placed on a water bath for a short time and stirred. A 2ml of the mixture was divided into two. To the first 1ml, few drops of freshly prepare Dragendoff's reagent was added and observed for formation of orange to brownish precipitate. To the second, one drop of Hager's reagent was added and observed for yellowish precipitate that is indicative of alkaloids.

Test for Free Anthraquinones

To 1mL of the HB cleanser® bitters, 10ml of chloroform was added and stirred. 10% ammonia solution was added to the stirred mixture. The presence of anthraquinone was detected by the presence of pink, red or violet colouration in the ammoniacal layer.

Test for Combined Anthraquinones

To 1 ml of the HB cleanser® bitters, 10 ml of aqueous sulphuric acid solution was added after which the mixture was boiled and filtered. The filtrate was extracted using 5ml of chloroform. To the extract, 10% ammonium hydroxide solution was added. The presence of pink or red or violet colour shows the presence of combined anthraquinones.

Test for Steroids/Triterpenoid

Lieberman-Burchard's test

To 1 ml of HB cleanser® bitters, 2ml of acetic anhydride was added. The mixture will be cooled in an ice followed by the careful addition of concentrated sulphuric acid down the side of the test-tube. A colour change from violet to blue to green indicates the presence of steroidal nucleus (aglycone portion of cardiac glycoside), a pink-red colour indicates triterpenoid nucleus.

Test for Cardiac glycosides

Salkwoski's test

To 1mL of HB cleanser® bitters, 2 ml of chloroform was added followed by few drops of concentrated sulphuric acid solution. An observation of a reddish-brown colouration at the interphase of the mixture indicates the presence of steroidal nucleus (aglycone portion of cardiac glycoside).

Test for Flavonoid

Shinoda Reduction Test: To a 1 mL HB cleanser® bitters, 3ml of chloroform was added for partitioning. To the chloroform layer, three pieces of magnesium chips were added followed by a few drops of concentrated hydrochloric acid. Appearance of an orange, pink or red to purple colour indicates the presence of flavonoids.

Sodium Hydroxide Test for Flavonoids: To a 1 mL HB cleanser® bitter, 5ml of distilled water was added followed by a few drops of 5% sodium hydroxide solution. The mixture was observed after which few drops of dilute hydrochloric acid was added followed by another few drops of 5% sodium hydroxide solution. An observation of a yellow colouration in the mixture which changes to normal on addition of dilute hydrochloric acid solution and reverts to yellow upon addition of another 5% sodium hydroxide indicates the presence of flavonoids.

Test for Tannins

To 1ml of HB cleanser® bitters, 5ml of distilled water was added. The mixture was heated, and two drops of ferric chloride solution was added afterwards. Formation of a blue black or green precipitate is an indication of the presence of tannins.

Test for Phlobatannin

To 1mL of HB cleanser® bitters, 5ml of distilled water was added and stirred. Few drops of 1% v/v hydrochloric acid solution were added. Deposition of a red precipitate was taken as a positive test for phlobatannins.

Test for Saponins

5ml of HB cleanser bitters was added to a test-tube. The test-tube was shaken and observed for frothing which persisted for 15 minutes which is characteristic of saponins.

Emulsion Test

To 5ml of HB cleanser bitters in a test-tube, 3ml of arachis oil was added. The test-tube was shaken vigorously and observed for the formation of a stable emulsion that does not form distinct layers in 5mins.

Test for Carbohydrate

Fehling's Test:

To 1 ml of the extract in a test-tube, 2ml of distilled water was added followed by a few drops of fehling's solution. The mixture was stirred and observed for the formation of red or green precipitate which indicates the presence of carbohydrate.

Molisch's Test

To 2 ml of HB cleanser bitters in a test-tube, 3 drops of Molisch's reagent was added followed by 1 ml of concentrated hydrochloric acid solution down the side of the test-tube. The formation of a purple layer at the interphase of the two layers indicates the presence of carbohydrates.

***In-vitro* Antioxidant Assay**

Total Phenol Determination:

The reaction mixture contained 200 µl of HB cleanser bitters®, 800 µl of freshly prepared diluted FolinCiocalteu reagent and 2 ml of sodium carbonate (7.5%). The final mixture was diluted to 7 ml with deionized water and kept in the dark at ambient conditions for 2h to complete the reaction. The absorbance was measured at 765 nm. Gallic acid was used as standard, and the results were expressed as gallic acid equivalents (GAE)[21].

Total Flavonoid Content

This was determined using aluminium chloride (AICI3). A volume of 0.1 ml of the sample was added to 0.3 ml distilled water followed by 0.03 ml of $NaNO_2$ (5%). After 5 min at 25ºC, 0.03 ml of $AICI_3$ (10%) was added. After a further 5 min, the reaction mixture was mixed with 0.2 ml of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. The results were expressed as quercetin equivalent (QE)[21].

Total antioxidant capacity

This was carried out according to the method described by Priesto *et al.*, which involves the reduction of molybdenum (VI) to molybdenum (V). HB cleanser bitters®, (0.3 ml) was added to 3 ml of combined solution of (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95ºC and allowed to cool to room temperature. The absorbance was measured at 695 nm against the blank reagent. The antioxidant activity was expressed as the equivalent of ascorbic acid[22].

2,2-Diphenyl, picryl-1, hydrazyl (DPPH) Scavenging Activity:

Three millilitres of the HB cleanser®bitter was put in the test tube and 1 ml of a methanol solution of DPPH (0.1 mM) was added. The mixture was kept in the dark at room temperature for 30 min and absorbance was measured at 517 nm against a blank. The same procedure was used for the ascorbic acid, as the standard. The following equation was used to determine the percentage of the radical scavenging activity of the extract.

$$\text{Scavenging effects (\%)} = 100 \times (A_0 - A_s)/A_0$$

Where A_0 is the absorbance of the blank and $A_s$is the absorbance of the sample[21].

Nitric Oxide Scavenging Activity:

The reaction mixture contained 2ml of sodium nitroprusside (10 mM) in 0.5 mL phosphate buffer (0.5 mL; pH 7.4). Various concentrations (12.5, 25, 50,100, 200, 400, 600, 800, 1000 µg/ml) of HB cleanser® bitters (0.5 mL) was added in a final volume of 3 mL.

After incubation for 60minutes at 37ºC, Griess reagent [α-napthyl-ethylenediamine (0.1%) and sulphanilic acid (1%) in H_3PO_4 (5%)] was added. The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with a-napthylenediamine was measured spectro-photometrically at 540 nm. Ascorbic acid was used as a positive control. The scavenging ability (%) of the nitric oxide was calculated using the formula:

$$\text{Scavenging effects (\%)} = 100x(A_0 - A_S)/A_0$$

Where A_0 is the absorbance of the blank and $A_s$absorbance of the sample[21].

Lipid Peroxidation Assay

Anti-Lipid peroxidation assay (TBARS) A modified thiobarbituric acid-reactive species (TBARS) assay[22] was used to measure the lipid peroxide formed, using egg yolk homogenate as lipid rich medium. Egg homogenate (0.5 mL of 10% v/v) and 0.1 mL of sample were added to a test tube and made up to 1mL with distilled water. Total 0.005 ml of $FeSO_4$ (0.07M) was added to induce lipid peroxidation and incubated for 30 min. Then 1.5 mL of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 mL of 0.8% (w/v) TBA in 1.1% sodium dodecyl sulphate and 0.5 mL 20% TCA were added and the resulting mixture was vortexed and then heated at 95ºC for 60 minutes. After cooling, 5.0mL of butanol were added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm. Inhibition of lipid peroxidation (%) by the extract was calculated using this formula:

$$(1\text{-}E/C) \times 100$$

Where

C is the absorbance value of the fully oxidized control.

E is (Abs532+TBA – Abs532-TBA).

Total Antioxidant Activity by Ferric Reducing Antioxidant Power Assay (FRAP):

The fresh ferric reducing antioxidant power assay(FRAP) reagent made up of 500 mL of acetate buffer (300 mM; pH 3.6), 50 mL of 2,4,6 – Tris (2-pyridyl) –s – triazine (TPTZ) (10 mM), with 50 mL of $FeC1_3.6H_2O$ (50 mM). The colorimetric determination was done at 593 nm and was observed for 12 minutes on 75 µL of each extract and 2 mL of FRAP reagent[21]. Vitamin C was used as standard.

Acute Toxicological Evaluation

Eighteen Wistar rats of average body weight of either sex was utilized for the acute toxicity. The animals were deprived of food a night prior to the study. The study was done in two phases of 9 animals each. The 9 animals in the first phase were sub-grouped into three groups of three animals each and were treated with 10,100 and 1000 mg/kg of the HB cleanser bitters® respectively and kept under close observation for twenty-four hours for any sign of toxicity or death. The 9 animals in the second phase were also subdivided into groups of three animals each and were exposed to 1600, 2900 and 5000 mg/kg respectively[23]. The dose equivalents of 10 mg, 100 mg, 1000 mg, 1600mg, 2900 mg, and 5000 mg were calculated from the relative density of the HB cleanser bitters®.

Experimental Design

The study used the method described by Nwidu *et al*., [24] and modified with the report of Sadeghi *et al*.,[25]. Twenty-eight male Wistar rats were randomly distributed into four groups, each comprising seven male rats. Group A, served as the negative control and received 5 ml/kg normal saline, groups C, D and E (the test groups) were administered with 1ml/kg, 1.03 ml/kg and 1.29 ml/kg of HB cleanser®bitters respectively, once daily through the oral route for twenty-eight days.

Assay of Serum Lipid Profile

The last dose of the bitters was administered on the 28th day. After an overnight fast and following light diethyl ether anaesthesia, blood samples were collected through the jugular vein of the animals and sent to the Department of Chemical Pathology research laboratory of the University of Port Harcourt Teaching Hospital for the analysis. The parameters assayed are total cholesterol, total triglyceride, high density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) using Randox kit (Randox lab. UK) and following the standard procedures as described by the manufacturers. The LDL-cholesterol was calculated using Friedwald's equation[26,27].

Statistical Analysis

All the data obtained were expressed as mean±SEM. Statistical analysis of data was done using one way analysis of variance (ANOVA), followed by post-hoc Tukey test with IBM SPSS version 21. *p*-values was considered statistically significant at a value of $p<0.05$.

RESULTS

Phytochemical Screening

Phytochemical screening revealed the presence of cardiac glycosides, carbohydrates, saponins and flavonoids. However, phlobatannins, steroids, tannins, purines alkaloids, free anthraquinones and combined anthraquinones were absent.

Table 1: Quantification of phytochemical constituents of HB cleanser bitters®.

Total Phenol (mg/100 g)	44.16±0.67
Total Flavonoid (mg/100 g)	84.92±0.98
Total Antioxidant Capacity (mg/100 g)	77.01±0.50

All values represent Mean ±SEM, n=3, significance=*$p<0.05$.

***In vitro* Antioxidant Activity**

There was a statistically significant difference ($p<0.05$) in antioxidant activity of the HB cleanser® bitters for the different antioxidant assay that was carried out. The total phenolic content, flavonoid content, and antioxidant capacity of the HB cleanser® bitters is as shown in Table 1. The DPPH scavenging activity of HB cleanser® bitters increased with increase in concentration at all the examined concentrations when compared to ascorbic acid (Figure 1).

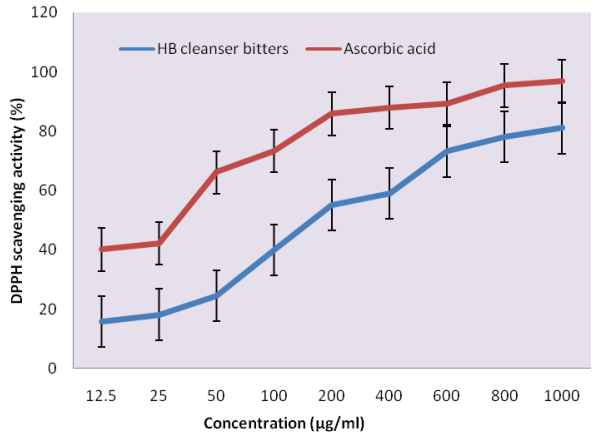

Figure 1: DPPH scavenging activity (% inhibition) of HB cleanser® bitters.

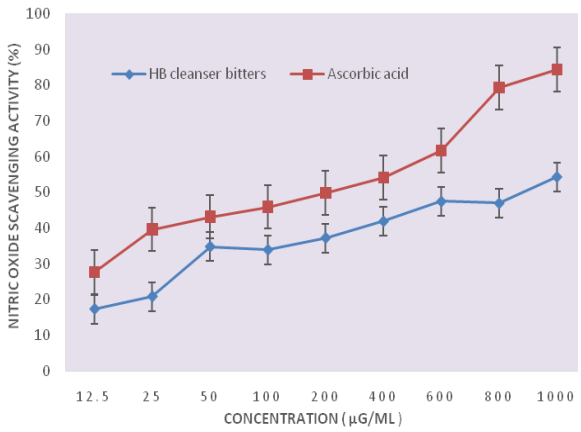

Figure 2: Nitric oxide scavenging activity (% inhibition) of HB cleanser® bitters.

The nitric oxide scavenging activity of HB cleanser® bitters showed an increase as the concentration increases. However, there was a decrease in the percentage inhibition at a concentration of 800 µg/ml when compared with ascorbic acid (Figure 2). There was a reduction in the ferric reducing power scavenging activity of HB cleanser® bitters at 200 µg/ml while an increase was observed at the other concentration when compared with ascorbic acid (Figure 3). Lipid peroxidation inhibitory activity of HB cleanser® bitters increased with increasing concentrations when compared to ascorbic acid (Figure 4).

Acute Toxicity

From the oral acute toxicity test, it was observed that the lethal dose (LD_{50}) of the HB cleanser® bitters is greater than 5000mg/kg since no death was recorded.

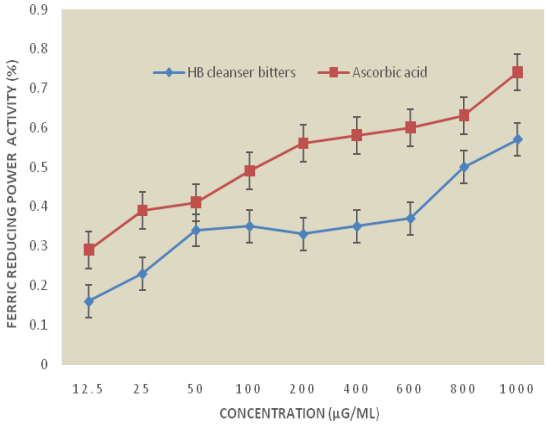

Figure 3: Ferric reducing power scavenging activity (% inhibition) of HB cleanser® bitters.

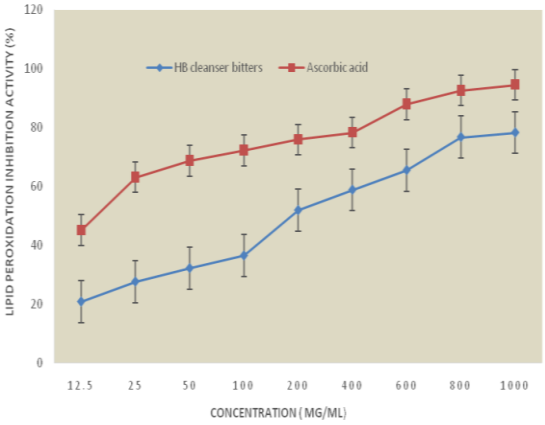

Figure 4: Lipid peroxidation inhibition activity (% inhibition) of HB cleanser® bitters.

Lipid Profile Assay Result

There was a statistically significant (p<0.05) difference in total cholesterol and HDL levels at 1 mL/kg of HB cleanser® bitters. The HDL level was also increased at 1 mg/mL, while a reduction in triglycerides level occurred at all the doses while there was a gradual increase in the LDL levels with increase in dose when compared to the control (Table 2).

DISCUSSION

The standardization and safety of herbal medicinal preparations have been a major concern for the regulatory and health authorities including the pharmaceutical manufacturing companies .Herbal preparations have been reported to contain phytoconstituents that convey antioxidant activities on them[28,29]. Antioxidants offer protection against the damages caused by free radicals in a biological system[30] due to the presence of phenolics and flavonoids which have redox reaction abilities through which free radicals such as singlet oxygen are scavenged[31]. Phytochemical evaluation of HB cleanser® bitters revealed the presence of cardiac glycosides, carbohydrates, saponins and flavonoids. The total phenol content of HB cleanser® bitters was44.16±0.67 mg/100g, flavonoids content was 84.92±0.98 mg/100g and antioxidant capacity was 77.01±0.50 mg/100g. The total phenol content serves as a measure of the antioxidant strength of aromatic and medicinal plants[32] but may not be significantly responsible for antioxidant activity in some medicinal plants[33]. Flavonoids have also been known to contribute to antioxidant effects of medicinal substances in physiological systems by scavenging free radicals[28,34].

Table 2: Evaluation of HB cleanser® bitters on lipid parameters.

Group	Dose (ml/kg)	Lipid Parameters (mmol/L)			
		TC	TG	HDL	LDL
Normal saline	5	2.34±0.17	1.04±0.19	0.60±0.10	1.27±0.15
HB cleanser	1	4.32±1.35*	0.90±0.12	0.62±0.13*	1.29±0.13
HB cleanser	1.03	2.20±0.19	0.86±0.18	0.44±0.18	1.33±0.21
HB cleanser	1.29	2.32±0.15	0.92±0.13	0.58±0.08	1.32±0.20

All values represent Mean±SEM, significance = *$p < 0.05$ when compared to the control; TC: Total cholesterol; TG: Total triglyceride, HDL: High density lipoprotein, LDL: Low density lipoprotein.

Total antioxidant capacity estimates the quantity of free radicals scavenged in each test sample[35] which serves as an indicator of the antioxidant potential of a medicinal substance[30,36]. DPPH assay evaluates the ability of substances to scavenge free radical or donate hydrogen and to evaluate antioxidant activity of substances including estimation of the number of antioxidants in biological systems[37]. DPPH is a free radical that stabilizes as a molecule once an electron or hydrogen atom is transferred to it[38]. Ascorbic acid is a powerful reducing agent which can donate hydrogen atom to nitrogen, oxygen radicals in a biological system[39].

In the present study, the DPPH scavenging activity of HB cleanser® bitters increased as the dose increased which was comparable to that of ascorbic acid. The higher the percent (%) inhibition of DPPH the lower the free radical scavenging activity and antioxidant power[40]. The IC_{50} is the concentration of the substrate that causes a 50% reduction in DPPH activity[41]. The IC_{50} of HB cleanser® bitters was 5.06 µg/ml while that of ascorbic acid (standard) was 1.62 µg/ml. The IC_{50} value is directly proportional to the potency of the sample. The lower the IC_{50} value, the more potent the substance or compound[42]. Nitric oxide (NO) is obtained from amino acid L-arginine by vascular endothelial cells, phagocytes, and certain cells of the brain. Nitric oxide is a free radical and a weak oxidant due to its unpaired electron and reacts with certain proteins and some free radicals. However, nitric oxide reaction with superoxide radical leads to the formation of an extremely reactive peroxynitrite anion ($ONOO^-$) which may precipitate unwanted toxicity[43]. Antioxidants obtained from natural sources such as medicinal plants often compete with nitric oxide for super oxide oxygen to inhibit the production of peroxynitrite which acts as an oxidant of biomolecules[44]. Nitric oxide inhibitory activity of HB cleanser® bitters increased with increase in concentration with an IC_{50} value of 7.98 µg/ml while that of ascorbic acid was 4.39 µg/ml which is comparable. Ferric reducing antioxidant activity measures the ability of a substance to donate an electron or hydrogen atom to break the free radical chain[45]. HB cleanser® bitters showed a good reducing power as it was able to reduce ferric ions (Fe^{3+}) to the ferrous ion (Fe^{2+}) with an IC_{50} of 1192.67µg/ml even though there was a decrease in reducing ability at the concentration of 200 µg/ml. This is in conformity with the report of Adebiyi *et al.*,[46] which stated that the scavenging activities of the spices used in their study increased with increasing concentrations and served as a reflection of the increased ability of the test constituents to easily contribute hydrogen atoms to the reactive free radical. The results of the antioxidant activity assays of HB cleanser® bitters can also be correlated to that of Shorinwa and Shatange[47] which reported that the leaves of *Smilax anceps* possessed potent antioxidant activity using similar assay protocols. Lipid peroxidation leads to cell damage while inhibition of peroxidation by antioxidants prevents or protects the cell from damage or destruction. Thus, lipid peroxidation may be considered as a biological marker for cell damage assessment[48]. Lipid peroxidation might alter the permeability of the cell membrane and influence metabolic processes including ion transport which may lead to increased reactive oxygen species expression[49]. Lipid peroxidation could also be used as an indicator of oxidative stress because of the hydroxyl free radical oxidation of polyunsaturated fats (PUFA) constituents of cell membranes[50].

HB cleanser® bitters exhibited a dose dependent inhibition of lipid peroxidation which increased consistently with increase in concentration. The findings of the lipid parameters evaluation revealed that the HB cleanser® bitters caused an increase in the low-density lipoprotein-cholesterol (LDL-C) while the total triglyceride, total cholesterol as well as the high-density lipoprotein-cholesterol (HDL-C) level were reduced even though the difference was statistically non-significant ($p>0.05$). This shows that HB cleanser® bitters relatively have hypo-lipidemic effects, while decreasing the total cholesterol and total triglyceride even though it decreased HDL-cholesterol and increased LDL-cholesterol levels. This result seems to give credence to the claim by bitters manufacturers that they have hypo-lipidaemic effect. There is evidence that a salient relationship exists between high serum cholesterol levels and the incidence of atherosclerosis and cardiovascular disease [51]. The observed hypolipidaemic effect of these herbal bitters is therefore a desired positive effect. This is in line with the result of the study carried out by Anionye *et al.*,[52] on the effects of Yoyo bitters on albino rats which stated that Yoyo bitters reduced the concentrations of total cholesterol and triglycerides. This is not to neglect the fact that the study revealed that HB cleanser® bitters increased the low-density lipoprotein-cholesterol when compared to the control. The observed antioxidant activities and hypo-lipidaemic effects might be attributed to the phenolic and flavonoid constituents of the bitters.

CONCLUSION

The study revealed that the antioxidant effect of the HB cleanser® bitters increases with increased

concentration. The consumption of HB cleanser® bitters should be done with caution as it was found to increase low density lipoprotein-cholesterol (LDL-C).

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AUTHORS CONTRIBUTION

SHORINWA OA: designed and supervised the study. **BENNETH VC:** carried out the study. Both authors drafted the manuscript while author. Final manuscript was read and approved by all authors.

DATA AVAILABILITY

The data and material are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

Authors hereby declare that no conflict of interest exists.

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