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Phytochemical, Biochemical and Biological Evaluation of Five Herbal Bitters Sold in Pharmacy Shops in Eastern Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study investigated the phytochemical constituents, antimicrobial and antioxidant properties of five herbal bitters and their potential effect on body weight**,** lipid profile, hematology, liver and kidney functions of albino rats.
Methods: Five brands of herbal bitters (Goko Cleanser®, Ruzu Bitter®, Yoyo Bitter®, Swedish Bitter® and Beta Cleanser®) were tested for the presence of phytochemical constituents. Antimicrobial activity was evaluatedby agar diffusion method. The weights of the animals were taken before treatment, and on day 7, 14, 21 and 28 post treatments with the herbal bitters. Blood levels of superoxide dismutase, catalase, glutathione peroxidase, cholesterol, triglyceride, HDL-cholesterol, PCV, haemoglobin, AST, ALT, ALP, urea and creatinine were measured.
Results: Glycosides, alkaloids, flavonoids, terpenoids, tannins, steroid, saponins, phenolic compounds were present while reducing sugar, amino acid and hydrogen cyanide were absent in the five bitters. All the five bitters showed moderate to potent antimicrobial activity against *Bacillus cereus, Staphylococcus aureus, Salmonella typhi, Escherichia coli*, *Aspergillus niger* and *Candida*

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albicans. There was a significant ($p<0.05$) decrease in body weight and a significant increase in catalase, SOD and glutathione peroxidase activities. There was also a significant reduction in total cholesterol and an increase in HDL. The PCV of the treated animals increased significantly while the haemoglobin was not affected significantly. The liver and kidney functions were not significantly altered.

Conclusion: Based on the results of this study, Goko Cleanser®, Ruzu Bitter®, Yoyo Bitter®, Swedish Bitter® and Beta Cleanser® possess antimicrobial and antioxidant properties and may help to reduce body weight and hypercholesterolemia.

Keywords: Herbal bitters; antioxidant; antimicrobial; cholesterol; body weight; lipid profile.

1. INTRODUCTION

Long before recorded history, herbs were exploited for medical purposes. Herbal medicine has been used to treat a variety of ailments in Africa since the arrival of Orthodox medicine. Modern pharmacology has its roots [1,2]. Approximately 80% of the world's population currently uses herbal medicines for various areas of their basic health care [3]. Millions of people are turning to traditional herbal medicines to prevent or treat a variety of ailments, and a sizable portion of pharmaceuticals distributed in community pharmacies now contain plant-derived drugs. This has resulted in the repackaging of "herbal bitters" and similar goods in a "orthodox style".

In Nigeria, the usage and sale of herbal remedies is on the rise. Cultural customs, religious beliefs, historical experiences, traditional ideas and behaviors, influence of friends and relatives, economic considerations, and deteriorating health are among the other reasons. Herbal medicines are effective in treating a variety of illnesses, including infectious ailments, hypertension, and others.

Herbal medications typically comprise a variety of pharmacologically active substances; in some cases, the exact elements that are necessary for the therapeutic effect are unknown [4]. Many herbalists feel that separated components have weaker therapeutic effects than entire plant extracts, although this is a claim that has to be proven. Many herbal therapeutic treatments on the market lack clinical data from high-quality randomized controlled studies, which is a typical criticism thrown at them. Although many herbal therapies have a scarcity of data, several randomized clinical trials (RCTs) have been published, and systematic reviews/ meta-analyses of these research have become routine [5–7].

Bitters are a category of chemical compounds taken from herbs and roots (medicinal plants) that have a bitter taste and operate to enhance the body's vital energy centers [1,8]. Bitters are made from narcotic components of tropical and subtropical plants and spices, as well as extracts of herbs and roots. To get the intended synergistic effect, the herbs are used in combination [9].

The name "bitters" as it is currently used refers to a beverage, usually alcoholic, that is flavored with herbal essences and has a bitter or bittersweet flavor. Herbal bitters are prescription and natural treatments extensively used in underdeveloped nations to alleviate indigestion and other stomach problems, as well as to treat a variety of disorders. In both developing and established countries where modern pharmaceuticals are mostly used in medicine, reports over the last two and a half decades have consistently revealed that herbal medicines (including herbal bitters) are becoming the most frequent form of alternative medicine [10]. Our historic diets were not devoid of bitter foods as they are now in most modern diets, thus there is a wish that we perceive the medicinal side of bitters in a new perspective in order to avoid what is known as "Bitter Deficiency Syndrome," which is a predisposing factor to many of today's maladies [11]. All of this makes research into the constituents and pharmacological effects of modern bitters both desirable and necessary.

Bitters are said to be useful in treating a wide range of allergy, metabolic, and immunological problems [12]. Bitters enhance the tone of tissues throughout the digestive tract and aid in the healing of damaged mucous membranes, in addition to their influence on digestive fluids that aid in good digestion [10]. Anti-inflammatory, antibacterial, and antifungal activities are also claimed. They can also aid with stomach inflammatory diseases. They are thought to repair and heal the mucosal lining of the G.I.T, particularly in the case of duodenal and gastric ulcers. Bitters are said to aid in the healing of piles and hemorrhoids, as well as improving

sexual function [13]. Bitters are claimed to offer anti-tumor qualities and to protect against colorectal cancer in particular. They improve blood circulation, kidney blood purification, blood pressure regulation through arterial dilatation, and kidney stone prevention. Bitters are also reported to aid in the digestion of fats and oils, as well as the proper functioning of the liver in excretion, and to lower stored fat (triglycerides) and cholesterol levels, giving it hypolipidemic characteristics [14]. They are supposed to remove extra body fat and promote healthy weight loss, as well as perform as a liver tonic and body detoxifier, as well as being hepatoprotective and increasing liver functions in general, as well as aiding in body detoxification [10]. Bitters have an effect on the pancreas and liver, assisting in pancreatic cell division and proliferation, as well as promoting the generation and release of pancreatic enzymes. Some people are even diagnosed with hypoglycemia [15]. However there is paucity of data establishing this claims, hence this study.

2. MATERIALS AND METHODOLOGY

2.1 Chemicals and Reagents

The herbal bitters (Yoyo bitter®, Swedish bitter®, Beta cleanser®, Ruzu bitter® and Goko cleanser®) were purchased from a reputable pharmacy shop in Enugu State, southeastern Nigeria.Merck, Germany, Sigma-Aldrich, Germany, British Drug Honses (BDH), UK, and Kieselgel GmbH, Germany, provided all analytical chemicals and reagents utilized in this investigation. Commercial test kits and products from Randox, UK, Biovendor, Czech Republic, TECO Diagnostics, USA, and Centronic GmbH, Germany were utilized in the assays. The Department of Microbiology, University of Nigeria, Nsukka, provided Gram-positive organisms (*Staphylococcus aureus* ATCC 9027, *Bacillus Cereus*), Gram-negative organisms (*Escherichia coli* ATCC 6538P, *Salmonella typhi*), fungal yeast *Candida albicans*, *and Aspergillus niger*. Mueller-Hinton Agar medium was used to keep them alive (Oxoid, UK). Each time, pure cultures that were 24 hours old were prepared.

2.2 Equipment

Centrifuge (Gallenkamp, Germany) Spectrophotometer (Jeol 400 MHz, Strathclyde Scotland University), water bath (Chikpas Instrument, Enugu), chemical balance (Gallenkamp, England), micro – pipettes (Perfect, USA), and capillary tube. Refrigerator (Haier thermocool, England), microscope (XSZ – 107BN, India).

2.3 Experimental Animals

The study employed adult rats of the winstar strain (75–100 g) of both sexes from the Department of Zoology and Environment Biology, University of Nigeria, Nsukka's animal holding unit. The animals were kept in regular settings (25 degrees Celsius and a 12-hour light/dark cycle).). The rats were given normal pellets twice a day (Grand Cereals Ltd, Enugu Nigeria) and had free access to clean drinking water In this investigation, the procedures for the care and use of laboratory animals were followed according to ESUT Ethical Committee clearance letter ESUT/AEC/0136/AP98 .

2.4 Microbiological Assay

Mueller-Hinton Agar (Oxoid, UK) was made according to the manufacturer's instructions, autoclaved, and dispensed in 12 x 12cm Petri dishes at a concentration of 20 mL per plate. Before usage, the set plates were incubated overnight to guarantee sterility.

The cup plate method was used to assess antibacterial activity. The diameter of the inhibition zone against test microorganisms was used to assess antimicrobial activity [16].

2.4.1 Qualitative phytochemical analysis

Various qualitative tests for tannins, flavonoids, alkaloids, saponins, hydrogen cyanides, phenols, steroids, tepenoids, reducing sugars, phenols, glycoside and reducing sugar were carried out using their standard methods [2]

2.5 Pharmacological Assay

2.5.1 Animal grouping

For this investigation, thirty albino rats were placed into six groups of five rats each. Group 1 is the control (untreated) receiving normal saline while groups 2, 3, 4, 5 and 6 represent the experimental groups that received I ml/kg daily of Goko Cleanser®, Ruzu bitter®, Yoyo bitter®, Swedish bitter®, and Beta cleanser® respectively. Each group was administered with the bitters or normal saline daily for 28days and then sacrificed and blood samples collected using cardiac acupuncture and key parameters assayed.

2.5.2 Weight reduction/incremental studies

All groups were weighed initially (before treatment) and reweighed on 7th, 14th, 21st, and 28th day post treatment.

2.6 Determination of the Enzymatic Antioxidants

2.6.1 Assay superoxide dismutase (SOD)

Superoxide dismutase activity and catalase were assayed while the level of the lipid peroxidation product, malondialdehyde (MDA), was measured spectrophotometerically to determine the glutathione concentration and to quantify the extent of lipid peroxidation (malondialdehyde) [17].

2.6.2 Lipid profile

Determination of the serum cholesterol (CHOL),serum triglycerides (TAG) and serum high density lipoproteins (HDL) were determined [18].

2.6.3 Haematological assay

Determination of the Packed Cell Volume (PCV) and Haemoglobin (HB) concentration were determined [19].

2.6.4 Liver function test activity

The activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)were assayed [20]

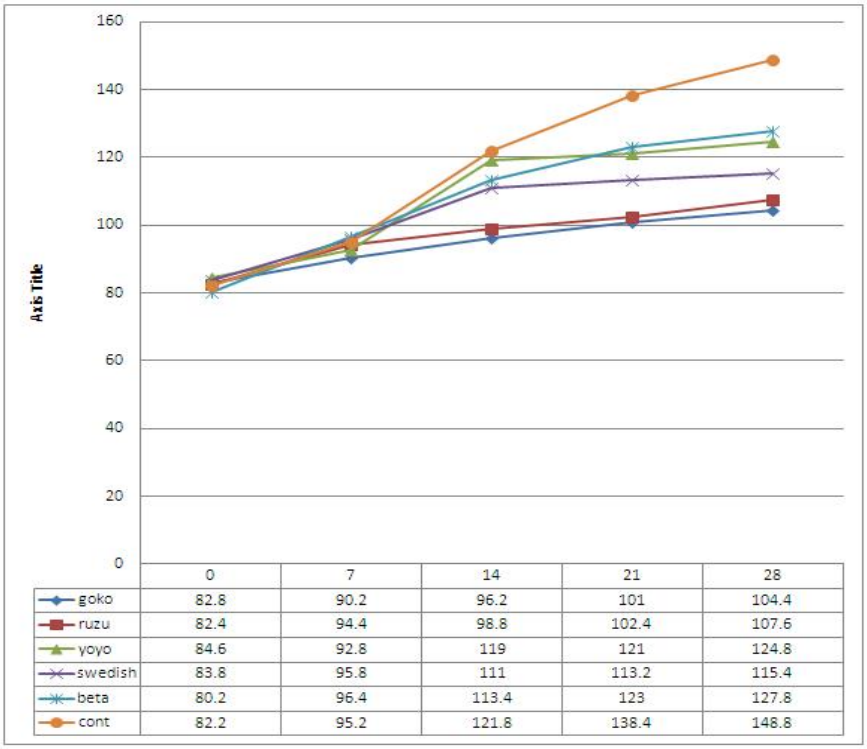
2.6.5 Kidney function test

The concentration of serum urea and serum creatinine were determined [17].

2.7 Statistical Analysis

For each group of animals, the data were expressed as Mean Standard Deviation. Using SPSS software, all of the aggregated data was statistically assessed. The threshold for statistical significance was fixed at $p< 0.05$.

3. RESULTS



	0	7	14	21	28
goko	82.8	90.2	96.2	101	104.4
ruzu	82.4	94.4	98.8	102.4	107.6
yoyo	84.6	92.8	119	121	124.8
swedish	83.8	95.8	111	113.2	115.4
beta	80.2	96.4	113.4	123	127.8
cont	82.2	95.2	121.8	138.4	148.8

Fig. 1. Effect of bitters on body weight of experimental animals in g

Key: goko= Goko Cleanser®; ruzu = ruzu Bitters®; yoyo = yoyo Bitters®; swedish = Swedish Bitters®; beta = Beta Bitters®; cont = Control

Table 1. Antimicrobial activity of the test herbal bitters against some clinical isolates

	Inhibition zone diameters (mm)					
Organism	**Yoyo Bitter®**	**Swedish Bitter®**	**Beta Cleanser®**	**Goko Cleanser®**	**Ruzu Bitter®**	**Control**
Salmonella typi	17 ± 0.3	23 ± 0.1	8 ± 0.1	11 ± 0.5	14 ± 0.3	15 ± 0.1
Staphlococcus aureus	15 ± 0.1	8 ± 0.4	18 ± 0.5	20 ± 0.3	13 ± 0.1	12 ± 0.3
Bacillus cereus	22± 0.3	8 ± 0.1	11 ± 0.2	10 ± 0.5	14 ± 0.1	10 ± 0.1
Echericha coli	10 ± 0.3	10 ± 0.3	18 ± 0.1	8 ± 0.3	13 ± 0.1	11 ± 0.3
Candida albicans	20 ± 0.1	11 ± 0.2	13 ± 0.1	20 ± 0.3	17 ± 0.5	15 ± 0.5
Aspergillus niger	22 ± 0.1	18 ± 0.3	11 ± 0.3	20 ± 0.1	15 ± 0.2	17 ± 0.1

Value are ± SEM, n = 5

Table 2. Qualitative phytochemical analysis of test herbal bitters

S/No	Phytochemical	Test	Yoyo bitter®	Swedish bitter®	Beta cleanser®	Goko cleanser®	Ruzu bitter®
1	Alkaloids	Dragendorrf reagent	++	++	+++	++	++
		Wagner reagent	-	-	-	+	++
		Mayer	-	++	-	-	-
2	Saponins	Frothing test	++	++	++	+++	++
		Emulsion test	-	-	-	-	-
3	Amino Acid	Ninhydrin test	-	-	-	-	-
		Biuret test	-	-	-	-	-
		Million's reagent	-	-	-	-	-
4	Flavonoids		+++	+++	+++	+++	++
5	Tannins		+++	+++	+++	+++	+++
6	Phenol Content	Ferric chloride	++	+++	++	++	++
7	Hydrogen Cyanide		-	-	-	-	-
8	Reducing Sugar		-	-	-	-	-
9	Glycosides	Fehling's test	++	++	+++	++	+++
10	Terpenoids		++	+	++	++	++
11	Steroids		++	++	++	++	+++

Key: +++ = Highly Present; ++ = Moderately Present; + = Slightly Present; - = Absent

Table 3. Antioxidant activity of the test herbal bitters

	SOD(iu/ml)	CATALASE(iu/ml)	GLU(iu/ml)	MDA(iu/ml)
Goko Bitters®	6.8064 ± 0.24	66.8792 ± 5.02	25.7964 ± 2.29	3.3 ± 0.60
Ruzu Bitters®	6.4456 ± 0.11	64.8582 ± 3.14	24.1196 ± 1.88	3.24 ± 0.27
Yoyo Bitters®	6.92522 ± 0.50	70.776 ± 4.41	28.1374 ± 0.42	3.38 ± 0.28
Swedish Bitters®	6.4528 ± 0.15	65.9628 ± 3.73	23.7032 ± 0.92	3.48 ± 0.24
Beta Bitters®	6.3004 ± 0.18	65.8984 ± 4.07	24.8956 ± 1.84	3.1 ± 0.46
Control	5.8344 ± 0.42	63.0688 ± 1.93	22.814 ± 1.23	3.42 ± 0.19

Value are iu/ml ± SEM, n = 5

Table 4. Effects of herbal bitters on lipid profile

	CHOLESTEROL	TG	HDL
Goko Bitters®	151.90 ± 9.62	121.61 ± 6.57	78.90 ± 5.92
Ruzu Bitters®	161.03 ± 21.51	126.64 ± 13.42	80.32 ± 8.95
Yoyo Bitter®	144.71 ± 13.80	117.21 ± 6.18	77.01 ± 5.40
Swedish Bitters®	153.11 ± 15.43	128.20 ± 12.01	84.01 ± 5.38
Beta Bitters®	161.68 ± 16.64	126.91 ± 5.88	88.83 ± 7.26
Control	169.81 ± 7.47	121.72 ± 7.30	72.81 ± 3.89

Value are md/dl± SEM, n = 5

Table 5. Effects of herbal bitters on liver enzymes and bilirubin levels

	ALT	AST	ALP	T. BIL	C. BIL
Goko	47.0 ± 10.3	25.0 ± 3.1	22.1 ± 2.3	0.51 ± 0.05	0.37 ± 0.03
Ruzu	50.0 ± 7.8	35.0 ± 9.0	21.2 ± 0.9	0.53 ± 0.10	0.35 ± 0.04
Yoyo	49.4 ± 8.6	26.8 ± 1.3	21.6 ± 1.1	0.56 ± 0.05	0.40 ± 0.01
Swedish	51.4 ± 5.4	28.2 ± 4.1	22.2 ± 1.7	0.55 ± 0.03	0.35 ± 0.03
Beta	43.6 ± 10.4	24.4 ± 2.9	20.8 ± 0.6	0.55 ± 0.05	0.39 ± 0.05
Cont	52.8 ± 5.6	31.8 ± 6.9	20.5 ± 0.6	0.55 ± 0.04	0.38 ± 0.03

Value are iu/ml ± SEM, n = 5

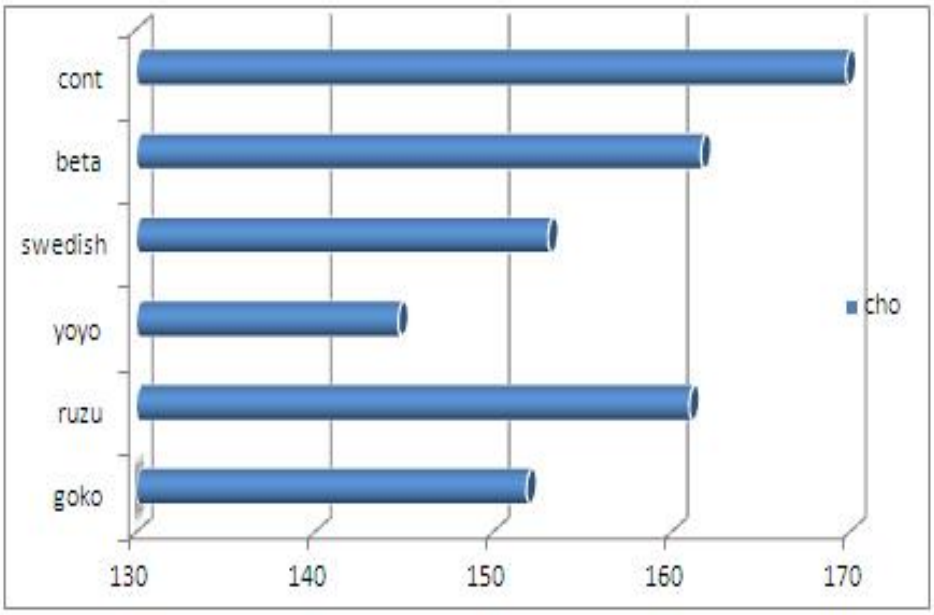


Fig. 2. Effect of the herbal bitters on total Cholesterol in mg/dl

Table 6. Effect of herbal bitters on kidney function

	UREA	CREATININE
Goko	24.9234 ± 1.1	0.5324 ± 0.08
Ruzu	25.5198 ± 2.1	0.6214 ± 0.06
Yoyo	25.7398 ± 3.6	0.5564 ± 0.05
Swedish	30.2384 ± 4.1	0.6082 ± 0.03
Beta	27.2708 ± 1.7	0.6232 ± 0.03
Cont	21.913 ± 2.3	0.6164 ± 0.06

Values are mg/dl ± SEM, n = 5

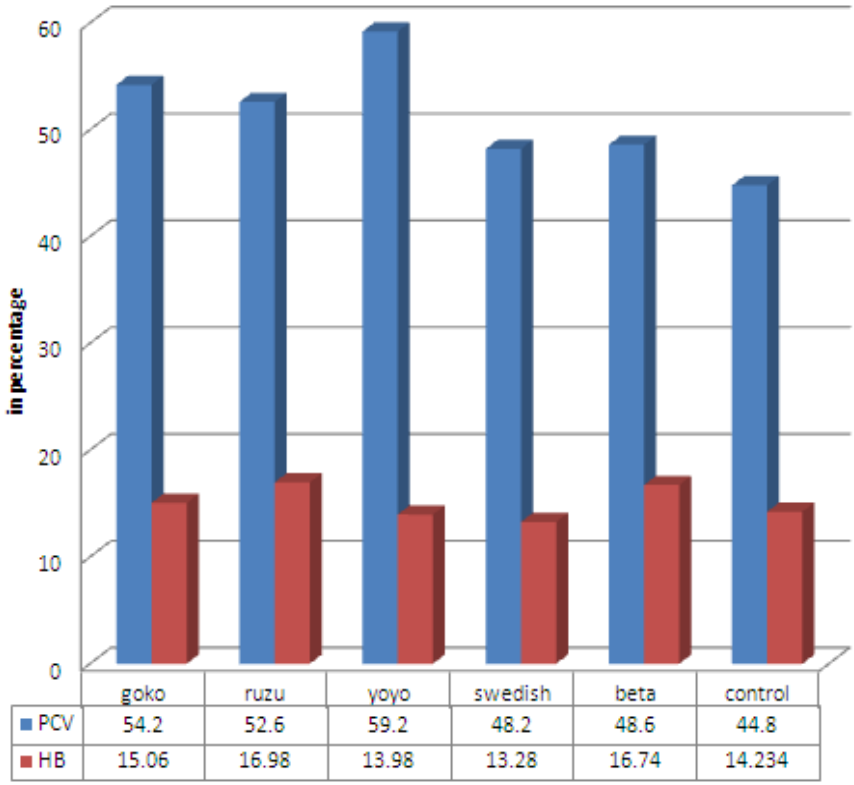


Fig 3. Effect of herbal bitters on packed cell volume and haemoglobin

4. DISCUSSION AND CONCLUSION

Preliminary results of the activities of antimicrobial agents such as plant active components are usually expressed *in vitro* as inhibition zone diameter of growth of test microorganisms around the sample. Any chemical that demonstrates activity with inhibition zone diameter of 8 mm and above is acceptable as being active [21]. All the samples showed activity against *Salmonella typhi, Staphylococcus aureus, bacillus cereus, E. coli, Candida albican and Aspergillus niger* with the IZD ranging from 8 - 23 mm as shown in Table 1.The antibacterial activity was in this order- yoyo biiter®> beta cleanser®>Ruzu bitter®> Swedish bitter® = Goko cleanser® while the antifungal activity followed this order -yoyo biiter®>Goko cleanser®>Ruzu bitter®> Swedish bitter®>beta cleanser®. The sample herbal bitters showed broad spectrum of antimicrobial activities by inhibiting the growth of both bacteria and fungi and could be used in treatment of infection caused by the test organisms.

Chemical molecules created during a plant's normal metabolic processes are known as phytochemicals. Alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, saponins, terpenes, and

terpenoids are among the compounds known as "secondary metabolites [22]. The qualitative evaluation of a herbal product's phytochemical contents is regarded as a crucial stage in medicinal plant research [23]. The presence of these secondary metabolites in plants likely explains their various uses as medicinal herbs. For example, its antioxidant constituents (hydrolysable tannins, phenolic acid, and flavonoids) have been shown to be effective in the prevention of coronary heart disease, cancer, and anti-carcinogenic and anti-mutagenic effects [24].

According to the result shown in Table 2, alkaloids, flavonoids, glycosides, phenol, tannins, saponins, steroid and terpenoids were present while amino acid, hydrogen cyanide and reducing sugar were absent in all the sample herbal bitters. Since this study reveals that the sample herbal bitters have appreciable amounts of secondary metabolites, their claimed medicinal uses is therefore not surprising. The most appropriate bitters can be simply given based on the recognized pharmaceutical properties of the discovered phytochemical constituents of herbal bitters, as well as the patient's medical needs/ailment and the phytochemical constituents' level in the herbal bitters.

At the end of the treatment period, the animals fed the sample herbal bitters had a significantly lower body weight growth than the control animals, as shown in Fig. 3. The highest weight reduction was seen with Goko cleanser® and Ruzu®, then Swedish® and Yoyo® and finally Beta Cleanser®. Weight increase has been linked to a variety of diseases as a risk factor.. Therefore the sample herbal bitters may be used to prevent such diseases thereby improving the quality of life.

Free radicals are to account for many of the ailments, including cancer [25], cardiovascular diseases [26], neural disorders [27], Alzheimer's disease [28], mild cognitive impairment [29], Parkinson's disease [30], alcohol induced liver disease [31], ulcerative colitis [32], aging [33]., and atherosclerosis [34]. Antioxidants can help protect you from free radicals if you eat a lot of them. Antioxidant-rich foods may play a key role in illness prevention, according to a growing body of data [35].

In this study, the levels of glutathione peroxidase, catalase and superoxide dismutase were determined. SOD and GSHPx are involved in the disposal of superoxide anions and hydrogen peroxide, and constitute the first line of cellular defense against oxidative harm. Hydroperoxides combine with reduced glutathione to create glutathione disulfide (GSSG) and the reduction product of hydroperoxide, which is catalyzed by glutathione peroxidase. The method for determining SOD used xanthine and xanthine oxidase to produce superoxide radicals, which then interacted with 2(-4- iodophenyl)-3(- 4 nitrophenol), – 5 phenyltetrazolium chloride (INT) to form a red formazan dye. The degree of inhibition of this process was used to determine the superoxide dismutase activity. The level of lipid peroxidation product was measured spectrophotometerically to determine lipid peroxidation. Malondialdehyde (MDA) is a byproduct of the lipid peroxidation process and is used as a lipid peroxidation biomarker.

Table 3 shows the results of catalase, glutathione peroxidase, and superoxide dimutase activities, as well as lipid peroxidation. There was a significance increase in superoxide dimutase activities in animals that have been fed Yoyo bitters®, Swedish bitters®, Goko®, Beta Cleanser® and Ruzu Bitter® in comparison to the control group at $p<0.05$.There was also an increase in catalase and glutathione peroxidase activities when compared with the control and the differences were statistically significant for animals on Yoyo bitters®. Except with Swedish bitter®, there was a decrease in MDA levels when compared to the control. The result of lipid peroxidation, malondialdehyde (MDA), is a measure of oxygen free radical levels.. Increased enzyme activity aids in the removal of oxygen free radicals [13]. The antioxidant ingredients (tannins, phenolic acid, and flavonoids) in these sample herbal bitters, as well as the reduction in oxygen free radicals, could help improve quality of life by preventing or delaying the onset of degenerative diseases.

Hyperlipidemia is a risk factor for cardiovascular illnesses such as myocardial infarction and atherosclerosis, which are leading causes of death and morbidity in the United States [36]. The lipid profile result (Table 4) demonstrates a reduction in the total cholesterol level for animals on Goko bitters® (151.90 ± 9.62), Ruzu bitters® (161.03 ± 21.51), Yoyo Bitters® (144.71 ± 13.80), Swedish Bitters® (153.11 ± 15.43) and Beta Cleanser®(161.68 ± 16.64) when compared with the control (169.81 ± 7.47) at $p<0.05$; and an increase in level of HDL in animal fed with Goko bitters® (78.90 ± 5.92), Ruzu bitters® (80.32 ± 8.95), Yoyo bitters® (77.01 ± 5.40), Swedish

Bitters® (84.01 ± 5.38) and Beta bitters®(88.83 ± 7.26) when compared with the control (72. 87 ± 3.89) at $p<0.05$.When the animals fed the sample bitters were compared to the control, there was no significant difference in triglyceride levels. Lowering total cholesterol and increasing HDL lowers the risk of hypercholesterolemia and hyperlipidemia, which can lead to coronary artery disease and other cardiovascular problems [37]. Therefore intake of bitters may reduce cardiovascular diseases.

The PCV of animals fed with the bitters increased significantly when compared with control. The difference in the PCV of animals fed with Swedish Bitters® and Beta Cleanser® was not significant. The difference in the HB level of animals fed with herbal bitters was also not significant except for Ruzu bitters®.

Liver function tests are used to reveal that an abnormality in the liver, such as inflammation or liver cell destruction, has occurred or is occurring. The alanine aminotransferase (ALT) and aspartate aminotransferase (AST), also known as the SGPT and SGOT, are the most often used indicators of liver (hepatocellular) injury. The alkaline phosphatase (ALP) test is the most commonly used to detect biliary blockage. These are enzymes that are typically located in liver cells but leak out and into the bloodstream when the cells are harmed. An elevation in their plasma concentrations indicates hepatic and cardiac damage [38]. There were no significant variations in the levels of these enzymes in animals fed the sample herbal bitters compared to the control, as shown in Table 5.This indicates that the sample bitters did not induce any liver damage when consumed.

Urea is a waste product that is produced when proteins are broken down. In most cases, urea is excreted in the urine. Because urea levels are influenced by protein intake and liver function, the test is frequently done in conjunction with a blood creatinine test, which is a more specific measure of kidney function. Creatinine is a waste product made by the muscles. Creatinine passes into the bloodstream, and is usually passed out in urine. A high blood level of creatinine indicates that the kidneys may not be working properly. Creatinine is usually a more accurate marker of kidney function than urea.

Table 6 shows the results of the renal function test. When comparing the treatment and control groups, there was a substantial increase in urea in the animals fed the sample bitters, but no significant variation in creatinine levels. Because creatinine levels are a more exact sign of renal function, it can be concluded that the experimental animals' kidneys were not harmed as a result of their use of herbal bitters.

In conclusion, the current findings suggest that moderate bitter intake can reduce body weight, total cholesterol, LDL-cholesterol, and lipid peroxidation while also increasing catalase activity.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

NOTE

The study highlights the efficacy of " Five Herbal Bitters " which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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