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Original Article

In vitro antioxidant and anti-inflammatory activities of selected polyherbal formulations sold in Nigeria

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Abstract

Background and aims: Nonsteroidal anti-inflammatory drugs (NSAIDs) or corticosteroid analgesics are recommended to alleviate or reduce pain. Regrettably, many of these drugs may induce bleeding, dyspepsia, cardiac complications, renal issues, and various short- or long-term adverse effects. Consequently, there has been an escalation in the endeavor to develop natural anti-inflammatory medications, driven by the growing number of individuals seeking natural therapies to manage their pain. The polyherbal formulations (Yoyo and Dr. Iguedo Goko bitters) have not assessed their antioxidant and anti-inflammatory activities; hence, this study "*in vitro* antioxidant and anti-inflammatory activities of selected polyherbal formulations sold in Nigeria."

Methods: The evaluation of antioxidant activity was performed using three assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, hydrogen peroxide (H_2O_2) scavenging activity, and ferric reducing antioxidant power. *In vitro*, anti-inflammatory efficacy was investigated using the human red blood cell (HRBC) membrane stabilization technique.

Results: The DPPH radical scavenging ability result was observed; the IC_{50} value of Yoyo bitters (40.63 ± 0.90 % at 250 µg/mL) was highest, while Dr. Goko bitters (3.26 ± 0.21% at 50 µg/mL) was lowest. Meanwhile, the positive control (ascorbic acid) had an IC_{50} value of 93.54 ± 0.57% at 250 µg/mL. The ferric-reducing antioxidant power of the samples was highest in Dr. Goko bitters, 1.96 ± 0.02 % at 250 µg/mL, and lowest in Dr. Goko and Yoyo bitters, 0.46 ± 0.01% at 100 and 50 µg/mL, respectively. The hydrogen peroxide free radical scavenging activity of the samples was highest in Yoyo bitters, 58.0.3 ± 0.60 % at 250 µg/mL, and lowest in Dr. Goko bitters, 7.28 ± 0.02 % at 50 µg/mL. The anti-inflammatory findings indicate that Yoyo bitters exhibited the highest percentage protection (89.46 ± 6.11%) at a concentration of 1000 µg/mL, while the lowest percentage protection (26.62 ± 1.13%) was observed at a concentration of 200 µg/mL. The reference standard diclofenac sodium has a percentage of 90.46 ± 1.44 at a concentration of 1000 µg/mL. Meanwhile, the poly-herbal formulations had the highest and lowest total phenolic concentrations in Yoyo bitters (97.52 ± 1.43 mg GAE/g at 250 µg/mL), and Dr. Goko bitters (47.3 ± 1.44 mg GAE/g at 50 µg/mL), respectively.

Conclusion: The findings suggest that the formulations might not have effective antioxidant and anti-inflammatory activities when compared with the reference standard drugs (ascorbic acid and diclofenac sodium), respectively.

Keywords: Antioxidant, Anti-inflammatory, Total phenolics, Poly-herbal formulation, Human health

Introduction

Humans have employed herbal medicines since the beginning of time (1). Modern medicine's inability to effectively treat some disorders has led to the beneficial alternative use of traditional herbal therapy (2). Herbal remedies are helpful in many nations for several ailments where conventional treatment has failed. Antioxidants have gained popularity recently, particularly those meant to protect against oxidative damage to the body and food product deterioration (3-7).

People of various ages might experience muscle inflammation. Over-the-counter (OTC) or prescription medications, such as nonsteroidal anti-inflammatory drugs (NSAIDs) or corticosteroid pain relievers, are advised to alleviate or minimize pain. Unfortunately, some of these medications might cause bleeding, indigestion, heart difficulties, renal problems, and other short- or long-term side effects. Some, like opioids, have a high potential for addiction. As a result, efforts to produce natural anti-inflammatory drugs have intensified as more individuals turn to natural remedies to control their pain (7).

Polyherbal formulations are marketed as "cure-all" medicines in Nigeria. Because of their health advantages, they are widely utilized and are common medications in many Nigerian homes. Ethnomedical herbal medicine has been proven effective for the prevention, diagnosis, control, and treatment of many diseases. According to Ukoha et al (8), the phytochemicals in these plants give

them their therapeutic potential.

Dr. Iguedos Goko Cleanser is a well-known poly-herbal medication that is used to treat a variety of ailments, including urinary disease, infertility, stabilizing menstruation, pain, aphrodisiac, worm expeller, malaria, stomach ulcers, and others. This medication works on bacteria and other organisms, which enhances low-density lipoprotein receptors and cholesterol, and also slows blood clotting that includes *Vernoni amygalina* (12%), *Saccharum officialis* (11.5%), *Alum sativum* (13%), and *Cajanus cajan* (11.5%) as active components (9).

An oral herbal remedy known as Yoyo Bitters Herbal Mixture (YBHM) is widely utilized throughout Nigeria. It belongs to the category of bitters made by Abllat Nigeria Company Limited, a local manufacturer of healthcare products. It is an herbal medicine introduced to the Nigerian market in 2003. The introduction of this product to Nigeria's medical industry has garnered widespread acceptance and usage among the general population. The National Agency for Food and Drug Administration and Control (NAFDAC) has officially acknowledged the drug as the initial authentic bitter produced in Nigeria, which does not contain alcohol, artificial coloring, or artificial preservatives. It has records for the treatment of, among other things, arthritis, obesity, hypertension, infertility, diabetes, and liver toning capability (10). The polyherbal formulations (Yoyo and Dr. Iguedo Goko bitters) have not been assessed for their antioxidant and anti-inflammatory activities. Therefore, this study aimed to evaluate the *in vitro* antioxidant and anti-inflammatory properties of selected polyherbal formulations sold in Nigeria.

Materıals and Methods

Chemicals

The analysis utilized albumin, trypsin, Tris-HCl, perchloric acid, casein, lipoxygenase, linoleic acid, lutein, rutin, β-carotene, and methanol. All other chemicals employed were of analytical grade.

Collection of herbal formulation samples

The two Nigerian Herbal formulations that were used in this study were purchased in Cynflac Pharmacy, Imgbi road, Yenagoa, Bayelsa State, namely Yoyo Cleanser Bitters (NAFDAC number: A7-1051L) and Dr. Iguedo Goko Cleanser® (NAFDAC registration number: A7-0804L). The samples were transported to the research laboratory of the Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, and after that, stored at room temperature before the experiment.

Preparation of samples

The samples (Goko and Yoyo) were evaporated to dryness at 40 °C using a constant-temperature water bath. The dry powder and the Standard (Ascorbic acid) were then constituted at 50, 100, 150, 200, and 250 µg/mL concentrations, as described by Joshi et al (11).

Fe^{3+} reducing power assay

A mixture was prepared by combining 2.5 mL of a phosphate buffer solution with a concentration of 0.2 M, 2.5 mL of a potassium ferricyanide solution with a concentration of 1%, and 1 mL of each of the test samples and the standard. Following 20 minutes of incubation at a temperature of 50 °C, a volume of 2.5 mL of trichloroacetic acid with a concentration of 10% was introduced into the mixture. The resulting solution was thoroughly mixed and then subjected to centrifugation at a speed of 3000 revolutions per minute for a duration of 10 minutes. The solution was prepared by mixing 2.5 mL of supernatant with 2.5 mL of distilled water and then adding 0.5 mL of a newly prepared ferric chloride solution with a concentration of 0.1%. The samples were subjected to a 10-minute incubation period, after which the absorbance at a wavelength of 700 nm was measured. The rise in absorbance seen in the samples was utilized as a quantitative indicator of their capacity for reduction.

DPPH scavenging activity

The capacity of the samples to give electrons or hydrogen was evaluated utilizing the DPPH methodology. To assess the radical scavenging ability, the experimental procedure involved the addition of samples to a 1 mM DPPH solution in ethanol, resulting in a purple-colored solution. Subsequently, the amalgamation was subjected to incubation under conditions devoid of light for 30 minutes, after which it was quantitatively assessed using spectrophotometry at a wavelength of 517 nm relative to a control consisting of distilled water. The observed reduction in absorbance of the sample indicates its ability to neutralize DPPH free radicals effectively.

The radical scavenging assay was quantified as percentage inhibition, which was computed by subtracting the absorbance of the test sample from the absorbance of the control sample and multiplying the result by 100.

The measurement of absorbance in the control group

$$\text{Radical scavenging assay (\% inhibition)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

H_2O_2 radical scavenging assay

The sample's capacity to remove hydrogen peroxide (H_2O_2) was assessed by mixing different amounts of the sample and a reference material with 0.6 mL of a phosphate buffer solution containing 40 mM H_2O_2. Following a period of incubation in a light-restricted environment for 10 minutes at ambient temperature, the samples were spectrophotometrically measured for absorbance at a wavelength of 230 nm. A blank solution consisting of phosphate buffer was employed, while a control solution containing 40 mM H_2O_2 in phosphate buffer was utilized.

The calculation of the radical scavenging percentage

was performed using the aforementioned formula.

$$\%\ inhibition = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} \times 100$$

Anti-inflammatory activity

The evaluation of the sample's anti-inflammatory effectiveness in a laboratory setting was conducted using the human red blood cell (HRBC) membrane stabilization technique described by Yesmin et al (12). Individuals in good health were subjected to blood sample collection. The blood samples were mixed with an equal amount of Alsever's solution, which had been sterilized. The mixture was homogenized entirely and then centrifuged at a rotating speed of 3000 revolutions per minute for 15 minutes. The pellet of red blood cells (RBCs) was washed three times using an isosaline solution. Then, a 10% suspension of HRBCs was created using the same isosaline solution. The sample was combined with an equivalent volume of phosphate buffer, followed by hyposaline and HRBC suspension in specific volumes: one milliliter, two milliliters, and 0.5 milliliters, respectively. Following incubation at 37 °C for 30 minutes, the reaction mixture was centrifuged at a speed of 3000 revolutions per minute for 10 minutes. The liquid portion was analyzed using spectrophotometry to measure its absorbance at a precise wavelength of 560 nm. Diclofenac served as the reference standard in the study. The hemolysis percentage was determined by assigning a value of 100% to the observed rate of hemolysis in the control group. The degree of protection or prevention of hemolysis was assessed by quantifying it using a percentage calculation based on the following formula:

$$\%\ of\ protection = 100 - \frac{Absorbance\ of\ the\ test\ sample}{The\ absorbance\ of\ the\ control} \times 100$$

Total phenolic content

Preparation of standard gallic acid for calibration curve

The quantification of the samples' overall phenolic contents was performed using the Folin-Ciocalteu colorimetric method, following the procedure described by Grigore et al (13), with specific modifications. To prepare the standard gallic acid solution, 10 mg of gallic acid was dissolved in 10 mL of methanol, resulting in a concentration of 1 mg/mL. The standard solution was used to prepare gallic acid solutions in methanol with varying concentrations (50, 100, 150, 200, and 250 µg/mL). To make a final volume of 10 mL, a solution containing 10% Folin-Ciocalteu reagent and a solution containing 7% Na_2CO_3 were combined in a ratio of 5 mL and 4 mL, respectively, for each concentration. The blue solution was thoroughly mixed and incubated for 30 minutes at 40 °C in a water bath. Afterward, the absorbance was measured at a wavelength of 760 nm relative to a blank sample. The experiments were performed three times, and the average absorbance values obtained at different concentrations of gallic acid were used to create the calibration curve.

Preparation of samples for total phenolic content

Different concentrations of the extracts (50, 100, 150, 200, and 250 µg/mL) were produced. The recommended experimental protocol for the standard gallic acid was adhered to, and precise absorbance measurements were recorded for each concentration of the extracts. Samples were generated three times for each analysis, and the calibration curve was created using the average absorbance value. This enabled the measurement of the phenolic content in the extracts. The quantification of the total phenolic content in the extracts was represented as milligrams of gallic acid equivalents (GAE) per gram of material in dry weight (mg/g). The overall phenolic levels in all samples were quantified using the equation C = c V/m. The variable C in this equation denotes the total phenolic content quantified in milligrams of gallic acid equivalents per gram of dry extract. The variable c represents the concentration of gallic acid obtained from the calibration curve, measured in milligrams per milliliter. V denotes the volume of the extract in milliliters, whereas m signifies the mass of the extract in grams.

Statistical analysis

The data were reported as the mean ± standard error of mean (SEM) based on three independent measurements. One-way analysis of variance (ANOVA) determined significant differences among the groups using the Statistical Package for the Social Sciences (SPSS 20) statistical analysis software.

Results

In vitro antioxidant results

The DPPH radical scavenging ability result was observed; the IC_{50} value of Yoyo bitters (40.63 ± 0.90% at 250 µg/mL) was highest, while Dr. Goko bitters (3.26 ± 0.21% at 50 µg/mL) was lowest. Meanwhile, the positive control (ascorbic acid) had an IC_{50} value of 93.54 ± 0.57% at 250 µg/mL, as shown in Table 1. The ferric-reducing antioxidant power of the samples was highest in Dr. Goko bitters, 1.96 ± 0.02 % at 250 µg/mL, and lowest in Dr. Goko and Yoyo bitters, 0.46 ± 0.01% at 100 and 50 µg/mL, respectively as shown in Table 2. The hydrogen peroxide free radical scavenging activity of the samples was highest in Yoyo bitters, 58.0.3 ± 0.60 % at 250 µg/mL, and lowest in Dr. Goko bitters, 7.28 ± 0.02 % at 50 µg/mL, as shown in Table 3.

Table 1. DPPH radical scavenging ability (%) of the poly-herbal formulations

Concentration (ug/mL)	Goko (% inhibition)	Yoyo (% inhibition)	Ascorbic (% inhibition)
50	3.26 ± 0.21^{a}	4.55 ± 0.35^{b}	23.85 ± 1.95^{a}
100	7.90 ± 0.46^{c}	10.25 ± 0.31^{e}	46.48 ± 0.44^{d}
150	12.30 ± 0.25^{e}	15.29 ± 0.99^{d}	64.94 ± 0.63^{c}
200	21.9 ± 0.39^{f}	24.02 ± 0.06^{a}	77.63 ± 0.54^{b}
250	35.6 ± 0.49^{d}	40.63 ± 0.90^{c}	93.54 ± 0.57^{e}

Note: Data are mean ± standard error of mean in triplicate determination. Means of the different superscript alphabets in the same column show no significant difference at 95% confidence levels ($P < 0.05$).

In vitro anti-inflammatory results

The anti-inflammatory (HRBC membrane stabilizing activity) findings indicate that Yoyo bitters exhibited the highest percentage of protection (89.46 ± 6.11%) at a concentration of 1000 µg/mL, while the lowest rate of protection (26.62 ± 1.13%) was observed at a concentration of 200 µg/mL. The reference standard diclofenac sodium has a percentage of 90.46 ± 1.44 at a concentration of 1000 µg/mL, as shown in Table 4. Meanwhile, the poly-herbal formulations had the highest and lowest total phenolic concentrations in Yoyo bitters (97.52 ± 1.43 mg GAE/g at 250 µg/mL) and Dr. Goko bitters (47.3 ± 1.44 mg GAE/g at 50 µg/mL), respectively shown in Table 5.

Discussion

The present study evaluated the antioxidant and anti-inflammatory activities of the polyherbal formulations using DPPH, reducing power assay, hydrogen peroxide scavenging activity, HRBC membrane stabilization activity, and total phenolic content. In DPPH radical scavenging ability, it was discovered that the Yoyo herbal formulation exhibited a higher potency, as evidenced by a $1C_{50}$ value of 40.63 ± 0.90% at a concentration of 250 µg/mL. In contrast, Dr. Goko bitters demonstrated the lowest potency with a value of 3.26 ± 0.21% at a 50 µg/mL concentration. In the experiment, ascorbic acid was utilized as the positive control, exhibiting an IC50 value of 93.54 ± 0.57% at a 250 µg/mL concentration. The findings of this work were inconsistent with the works of Grigore et al (13) and Enenebeaku et al (14).

Reducing power assay is an essential parameter for evaluating the antioxidant activity of the poly-herbal formulations. The samples in this study exhibited varying levels of ferric-reducing antioxidant power. Among these formulations, Dr. Goko bitters had the maximum antioxidant power at a concentration of 250 µg/mL, with a value of 1.96 ± 0.02%. On the other hand, Dr. Goko and Yoyo bitters exhibited the lowest antioxidant power at concentrations of 100 and 50 µg/mL, with values of 0.46 ± 0.01% (Table 2). The observed enhancement in reducing power within the poly-herbal formulations suggests certain constituents possess electron-donating properties. These constituents can engage with free radicals, converting them into more stable molecules, effectively terminating radical chain reactions. The formulations exhibited an increase in reducing power as the concentration rose. The findings of this investigation are consistent with the research conducted by Rahman et al (15). Hydrogen peroxide exhibits the ability to traverse cellular membranes and potentially engage in oxidation reactions with various substances. The poly-herbal formulations exhibited varying levels of hydrogen peroxide free radical scavenging activity. Among the formulations, Yoyo bitters demonstrated the maximum activity, with a percentage of 58.0.3 ± 0.60 at a concentration of 250 µg/mL. On the other hand, Dr. Goko bitters displayed the lowest activity, with a percentage of 7.28 ± 0.02 at a concentration of 50 µg/mL, as indicated in Table 3. The findings presented in this study align with the results reported by Subhashini et al (16).

The Poly-herbal formulations can stabilize cell membranes by preventing hypotonicity, indicating their potential anti-inflammatory capabilities (17). The present study investigated the membrane stabilizing activity of the polyherbal formulations on HRBCs. The results revealed that Yoyo bitters exhibited the highest percentage of protection, with a value of 89.46 ± 6.11% at a concentration of 1000 µg/mL. Conversely, the lowest protection rate was observed at a concentration of 200 µg/mL, with a value of

Table 2. Ferric reducing antioxidant power of the poly-herbal formulations

Concentration (µg/mL)	Goko (%)	Yoyo (%)	Ascorbic acid (%)
50	0.51 ± 0.01^{c}	0.46 ± 0.01^{e}	0.83 ± 0.02^{d}
100	0.46 ± 0.01^{d}	0.82 ± 0.05^{c}	1.0 ± 0.38^{a}
150	1.45 ± 0.07^{e}	1.49 ± 0.02^{d}	1.62 ± 0.02^{b}
200	1.80 ± 0.04^{a}	1.84 ± 0.03^{b}	1.88 ± 0.003^{c}
250	1.96 ± 0.02^{b}	1.92 ± 0.003^{a}	1.99 ± 0.003^{e}

Note: Data are mean ± standard error of mean in triplicate determination. Means of the different superscript alphabets in the same column show no significant difference at 95% confidence levels ($P < 0.05$).

Table 3. Hydrogen peroxide free radical scavenging activity of the poly-herbal formulations

Concentration (µg/mL)	Goko % inhibition	Yoyo % inhibition	Ascorbic acid % inhibition
50	7.28 ± 0.02^{c}	8.39 ± 0.16^{a}	12.65 ± 0.13^{b}
100	12.25 ± 0.11^{d}	13.79 ± 0.20^{b}	35.86 ± 0.29^{c}
150	22.2 ± 0.65^{e}	23.71 ± 0.36^{e}	56.13 ± 0.94^{e}
200	37.85 ± 0.44^{f}	38.62 ± 0.13^{d}	81.72 ± 0.53^{d}
250	53.12 ± 0.48^{a}	58.03 ± 0.60^{f}	98.17 ± 0.47^{a}

Note: Data are mean ± standard error of mean in triplicate determination. Means of the different superscript alphabets in the same column show no significant difference at 95% confidence levels ($P < 0.05$).

Table 4. Human Red Blood Cell membrane stabilizing activity (% protection) of the poly-herbal formulation

Concentration µg/mL	Goko % protection	Yoyo % protection	Diclofenac % protection
200	28.16 ± 1.22^{c}	26.62 ± 1.13^{d}	63.18 ± 1.03^{b}
400	42.74 ± 1.22^{d}	43.46 ± 0.38^{e}	75.77 ± 0.95^{a}
600	53.24 ± 0.80^{e}	54.35 ± 3.9^{a}	80.01 ± 1.26^{c}
800	62.37 ± 1.64^{a}	69.96 ± 0.95^{b}	56.88 ± 2.32^{e}
1000	79.25 ± 1.04^{b}	89.46 ± 6.11^{c}	90.46 ± 1.44^{d}

Note: Data are mean ± standard error of mean in triplicate determination. Means of the different superscript alphabets in the same column show no significant difference at 95% confidence levels ($P < 0.05$).

Table 5. Total phenolic content (mg GAE/g) of the poly-herbal formulation

Concentration µg/mL	Goko (mg GAE/g)	Yoyo (mg GAE/g)
50	47.3 ± 1.44^{a}	47.52 ± 1.43^{d}
100	51.65 ± 0.33^{b}	52.21 ± 0.33^{e}
150	58.51 ± 0.24^{d}	59.85 ± 0.19^{c}
200	75.5 ± 3.13^{e}	80.25 ± 0.46^{e}
250	87.33 ± 1.44^{c}	97.52 ± 1.43^{b}

Note: Data are mean ± standard error of mean in triplicate determination. Means of the different superscript alphabets in the same column show no significant difference at 95% confidence levels ($P < 0.05$).

26.62 ± 1.13%. The diclofenac sodium reference standard has a concentration of 90.46 ± 1.44% at 1000 µg/mL. The aforementioned in vitro methodology showed enhanced efficiency in time management, adaptability, and overall convenience. Based on the findings of this experiment, it may be inferred that the poly-herbal formulations exhibit favorable membrane stability, demonstrating effective anti-inflammatory properties. The findings presented here are consistent with previous research by Kumar et al. (17) and Rajalakshmi et al. (18).

The quantity and placement of hydroxyl groups primarily determine the efficacy of polyphenols in inhibiting free radical oxidation. These compounds, identified as the key agents responsible for scavenging free radicals and the antioxidant properties observed in plants (19-21), play a crucial role in the potential of poly-herbal formulations as free radical scavengers (22). In our study, the herbal formulations demonstrated varying levels of total phenolic content. Yoyo bitters exhibited the highest concentration at 97.52 ± 1.43 mg GAE/g at 250 µg/mL.

On the other hand, Dr. Goko bitters had the lowest concentration, measuring 47.3 ± 1.44 mg GAE/g at a concentration of 50 µg/mL, as indicated in Table 5. The findings align with the research conducted by (23,24) on the overall phenolic composition of infusions derived from several members of the Lamiaceae family, such as lemon balm, common basil, sage, peppermint, and others. The results obtained in this study exhibited a correlation with the findings reported by Khomdram and Singh (21).

Conclusion

The DPPH technique, ferric reducing power, and H2O2 assays showed that the analyzed samples (Yoyo and Goko) had lower antioxidant activities than the reference standard (Ascorbic acid). Meanwhile, Yoyo bitters had the highest anti-inflammatory impact on Human Red Blood Cell membrane stabilization compared to Goko bitters. However, the reference standard diclofenac sodium has a higher percentage when compared with the analyzed samples. Yoyo bitters had the highest total Phenolic content when compared with Goko bitters. The research suggests that the formulations are ineffective in antioxidant and anti-inflammatory activities compared with the reference standard drugs (Ascorbic acid and diclofenac sodium). Also, further investigation is required for the bioactive compounds and cytotoxicity of the polyherbal formulations.

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Authors' Contribution

Conceptualization: Odangowei Inetiminebi Ogidi.
Data curation: Odangowei Inetiminebi Ogidi.
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Competing Interests

The authors declared no conflict of interest in the manuscript.

Ethical Approval

This study was approved by the Research and Ethics Committee of the Department of Biochemistry, Faculty of Basic Medical Sciences, Bayelsa Medical University, Yenagoa, Bayelsa State, Nigeria, with a Reference Number FBMS/AD/BCH/REC/29/01.

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