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Original Article

Antioxidant and anti-inflammatory properties of selected polyherbal preparations: Oroki herbal, Swedish bitters and Yoyo bitters

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ABSTRACT

Objective: Polyherbal preparations are extracts obtained from different medicinal plants. These herbal preparations are orally administered as a therapy against numerous oxidative stress and inflammation mediated diseases. This study was designed to evaluate the antioxidant and anti-inflammatory properties of Oroki herbal, Swedish bitters and Yoyo bitters using *in vitro* models. **Methods:** Antioxidant studies were conducted using total antioxidant capacity (TAC), thiobarbituric acid reactive substances (TBARS), ferric reducing antioxidant power (FRAP), and 1'1'-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays while anti-inflammatory studies were performed using human red blood cell (HRBC) membrane stabilization and anti-denaturation of bovine serum albumin (BSA) assays. **Results:** Data showed that Oroki herbal had the highest TAC, inhibition of TBARS, FRAP absorbance range, DPPH scavenging activity while Swedish bitters had the highest stabilization of hypotonicity-induced hemolysis of HRBC and inhibition of heat-induced BSA denaturation when compared with other herbal preparations. **Conclusion:** Findings from this study indicated that Oroki herbal exhibited the highest antioxidant activity while Swedish bitters had the highest anti-inflammatory property *in vitro*. Furthermore, the medicinal properties of these polyherbal preparations could be attributed to their antioxidant and anti-inflammatory activities that could mitigate against oxidative stress and inflammation mediated diseases.

KEY WORDS: Anti-inflammation, herbal remedy, polyherbal formulation

INTRODUCTION

A major source of active compounds is medicinal plants that are thought to be effective with minimal side effects than conventional drugs [1]. In some countries, particularly developing ones, individuals that play an active role in their health care have chosen herbal medicine as a common choice in self-therapy against oxidative stress and inflammation-mediated diseases [2,3]. More than three-quarter of the world's population is increasingly diverting to herbal medicine due to its capacity to reduce ailments at an economical rate, and the trend is increasing globally [4].

Herbal medicines which are alternatively called botanical medicine or phytomedicine refer to herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other plant materials as active ingredients [5]. According to the World Health Organization, herbal medicines among the general population are medications prepared from one or more herbs or plant parts including roots, stem, bark, seeds, and leaves [6]. In Nigeria, herbal preparations, including Oroki herbal, Swedish Bitters, and Yoyo Bitters have become a slight medical option in many Nigerian homes due to their easy access and affordability.

Oroki herbal is a polyherbal preparation which comprises *Sorghum bicolor* stem, *Khaya grandifoliola* bark, *Cassia sieberiana* root, *Staudtia stipitata* root, *Alstonia congensis* bark, *Ocimum basilicum* leaves, *Mangifera indica* leaves, *Cyathula prostrata* leaves, *Securidaca longepedunculata* root, and *Saccharum officinarum* stem. This mixture has been formulated for pile, dysentery, constipation, diarrhea, blood stooling, waist and stomach pain, withdrawn protruding rectum, deworming, dysmenorrhea, and men turgidity. Swedish bitters is a polyherbal mixture which comprises *Aloe vera*, rhubarb roots, zedoary roots, manna, theriac venez S. Opio, angelica roots, myrrh, carline thistle roots, camphor, and Saffron. This formulation has been used internally as well as externally and it promotes biliary, pancreatic and gastric secretion, disinfects and soothes the digestive and intestinal tract, relieves bloating, flatulence, gas, cramps, and nausea. It is also known to encourage toxin elimination, tonify and regenerate the skin as well as serve as a gentle laxative among other functions. Yoyo bitters, also called Yoyo cleanser bitters, is a polyherbal mixture comprising

A. vera, *Acinos arvensis*, *Citrus aurantifolia*, *Chenopodium murale*, and *Cinamomum aromticum*. It was formulated to scavenge free radicals in the body, remove harmful toxins in the body, enhancement of effective function of secretory glands, improving liver functions and facilitating elimination of cholesterol, sugar, and triglycerides.

Some of these herbal preparations' healing properties have not been well researched, and consequently, their mechanism of action is poorly understood despite their acclaimed medicinal benefits. However, recent studies have demonstrated the need to subject some of the herbal preparations through a scientific validation process to ascertain their health benefits [7,8]. Therefore, this study was designed to evaluate the antioxidant and anti-inflammatory properties of the abovementioned polyherbal preparation: Oroki herbal, Swedish bitters and Yoyo bitters with the rationale to proffer a scientific explanation for the local use of these formulations.

MATERIALS AND METHODS

Herbal Preparation

Herbal preparations of Oroki herbal, Swedish bitters and Yoyo bitters were purchased from a Pharmacy Store in Lagos State, Nigeria.

In Vitro Antioxidant Assays

Total antioxidant capacity (TAC)

TACs of Oroki herbal, Swedish bitters and Yoyo bitters were determined using modified phosphomolybdate method as described by Prieto *et al.* [9]. An aliquot of 0.1 ml 0.1% herbal preparation was combined with 1 ml working reagent (0.6 M sulfuric acid, 28 mM sodium dihydrogen phosphate, and 4 mM ammonium molybdate). The standard consisted of 0.1 ml of varying concentrations of 1 mg/ml ascorbic acid in distilled water with 1 ml working reagent. Solutions were incubated in a water bath (Uniscope SM801A; Surgifield Medicals, Okehampton, England, UK) at 95°C for 90 min and cooled at room temperature. Absorbance was measured at 695 nm using a double beam ultraviolet (UV)-visible spectrophotometer (Model T80; PG Instruments, Lutterworth, England, UK) against a blank solution containing 0.1 ml distilled water and 1 ml working reagent incubated under the same condition. TAC was expressed as equivalents of ascorbic acid per ml sample. All determinations were performed in triplicate.

Thiobarbituric acid reactive species

Thiobarbituric acid reactive substances (TBARS) assay was carried out using the method described by Hodges *et al.* [10]. Varying concentrations of gallic acid were prepared and made up to 1.0 ml in test tubes using distilled water. Similarly, samples of Oroki herbal, Swedish bitters and Yoyo bitters were prepared and made up to 1.0 ml using distilled water. To these test solutions, 2 ml 20% (w/v) trichloroacetic acid, and 2 ml 0.67% (w/v) of thiobarbituric acid solutions were added. Another set of tubes containing the above reagents without any sample was used as a blank. The test tubes were placed in a boiling water bath for 10 min, cooled and centrifuged at 3000 rpm for 20 min. Absorbance of supernatants was measured at 552 nm using the double beam UV-visible T80 spectrophotometer (PG Instruments). This assay was carried out in triplicate, and percentage inhibition was calculated according to the formula below:

$$\% \text{ inhibition} = Abs_{control} - Abs_{sample}/Abs_{control} \times 100$$

$Abs_{control}$ = The absorbance without sample,
Abs_{sample} = The absorbance of herbal extract or standard.

Ferric reducing antioxidant potential

Ferric reducing assay was done using the method described by Benzie and Strain [11]. A solution of 2.5 ml 0.2 M phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide (1% w/v) was added into test tubes containing 1 ml herbal preparations at different concentrations. The reaction mixtures were incubated at 50°C for 30 min, followed by the addition of trichloroacetic acid (2.5 ml 10% w/v). The reaction mixtures were centrifuged at 3000 rpm for 10 min, and the upper layer of the solution was collected. A volume of 2.5 ml supernatant solution was mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (0.1% w/v). The absorbance was then measured at 700 nm against blank sample spectrophotometrically. Ascorbic acid was used as the standard.

1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging activity was determined as described by Molyneux [12] with slight modifications. 1 ml of 0.2 mM DPPH prepared with methanol was added to 2.5 ml solution of varying concentrations of herbal preparation and gallic acid as standard at different concentrations of 25, 50, 100, 250, and 500 μg/ml and allowed to react at room temperature in darkness for 30 min. 1 ml of 0.2 mM DPPH and 2.5 ml methanol served as a control. This assay was carried out in triplicates for each concentration. Absorbance of the resultant mixture was measured spectrophotometrically at 517 nm. Percentage inhibitions of the herbal preparations and the standard were calculated using the formula below:

$$\% \text{ inhibition} = Abs_{control} - Abs_{sample}/Abs_{control} \times 100$$

Percentage inhibition indicates the capacity of fractions to inhibit reactive oxygen species, and the concentration of sample required for 50% inhibition was determined and expressed as an IC_{50} value.

In Vitro Anti-inflammatory Assays

Erythrocyte stabilization assay

Effects of Oroki herbal, Swedish bitters and Yoyo bitters on hypotonicity-induced hemolysis of human red blood cell membrane (HRBC) were carried out in accordance to the

protocol described by Oyedapo [13]. Fresh human whole blood (5 ml) was collected using a 5 ml syringe and was immediately transferred to a bottle containing ethylenediaminetetraacetic acid as an anticoagulant. Whole blood was centrifuged for 10 min at 3000 rpm, and supernatant (plasma and leukocytes) was carefully removed while the packed red blood cells were washed in freshly prepared 0.9% w/v NaCl 3 times. 10% HRBC membrane was then resuspended in 0.9% w/v NaCl as stock. The assay mixture was subsequently prepared as 1 ml sodium phosphate buffer (pH 7.4, 0.15 mol/L), 2 ml 0.36% w/v NaCl, 0.5 ml stock HRBC suspension (10%, v/v) with 0.5 ml diclofenac sodium as standard drug or herbal preparations of varying concentrations in test tubes. For the control, distilled water replaced NaCl (0.36%, w/v) to induce 100% hemolysis. The different test tubes were incubated at 56°C in a water bath (Uniscope SM801A; Surgifield Medicals) for 30 min and then centrifuged at 5000 rpm. The hemoglobin content in each tube was estimated spectrophotometrically at 560 nm. The experiment was performed in triplicates for all the test samples.

Anti-denaturation of bovine serum albumin (BSA)

Anti-denaturation of BSA was assayed using a modified method of Ramalingam *et al.* [14]. The reaction mixtures contained 50 μl various studied concentrations of the standard drug (diclofenac sodium), herbal preparations and methanol as a control in test tubes. BSA (450 μl, 5% w/v) was added to the above test tubes and subsequently incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the test tubes, 2.5 ml phosphate buffered saline (pH 6.3) was added to each tube. Absorbances of these solutions were measured spectrophotometrically at a wavelength of 660 nm. The experiment was performed in triplicate. The IC_{50} was estimated using linear regression equation.

Statistical Analysis

Statistical analysis and graphical presentations were performed using GraphPad Prism® 7.0 software. It was used to determine the difference between means of TAC through one-way analysis of variance while computing the IC_{50} values for antioxidant and anti-inflammatory assays. Values were reported as a mean ± standard error of the mean of triplicate readings.

RESULTS

Data from Table 1 shows that Oroki herbal represented a significantly high (68 ± 0.08 mg ascorbic acid equivalent [AAE]/g, $P < 0.05$) TAC followed by Yoyo bitters (0.42 ± 0.04 mg AAE/g) and Swedish bitters (0.39 ± 0.02 mg AAE/g). Figure 1 also presents that 100-500 μl v/v% gallic acid, Swedish bitters, Yoyo biters and Oroki herbal inhibited TBARS in a concentration-dependent manner. The IC_{50} values for TBARS inhibition of gallic acid, Oroki herbal, Yoyo bitters and Swedish bitters were 83.05, 187.8, 274.3, and 555.8 μl v/v%, respectively [Table 2]. Further study showed that the ferric reducing capacities of 25-500 μl v/v% ascorbic acid, Oroki herbal, Swedish bitters and Yoyo bitters at 700 nm exhibited absorbance ranges of 0.69-2.32, 0.42-0.48, 0.35-0.55, and 0.28-0.36, respectively [Figure 2]. In addition, data in Figure 3 shows that 25-500 μl v/v% gallic acid, Oroki herbal, Yoyo bitters and Swedish bitters scavenged DPPH radical in a concentration-dependent manner. The IC_{50} values

Table 1: Effect of Swedish bitters, Yoyo cleanser bitters and Oroki herbal on TAC

0.1 ml/ml sample (v/v%)	Mean±SD (mg AAE/g)
Swedish bitters	0.39±0.02
Yoyo cleanser bitters	0.42±0.04
Oroki herbal mixture	0.68±0.08*

*$P<0.05$. AAE: Ascorbic acid equivalent, SD: Standard deviation, TAC: Total antioxidant capacity

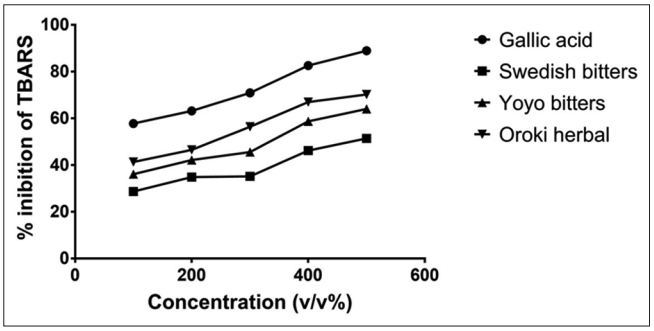

Figure 1: Effects of Oroki herbals, Swedish bitters and Yoyo bitters on the formation of thiobarbituric acid reactive species

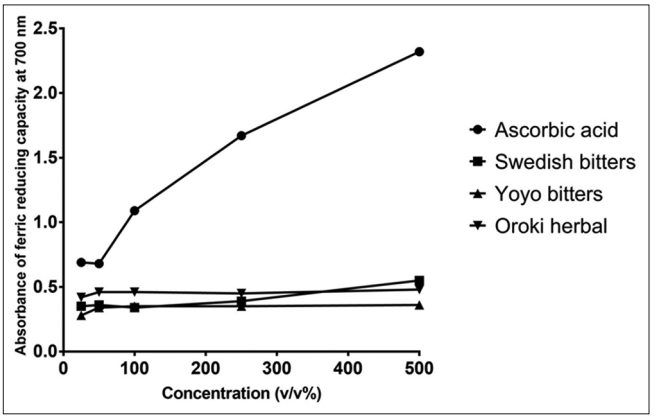

Figure 2: Ferric reducing antioxidant potentials of Oroki herbals, Swedish bitters and Yoyo bitters

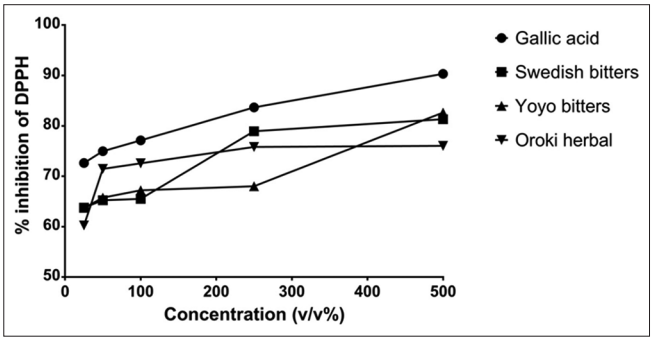

Figure 3: Percentage inhibition of 1'1'-diphenyl-2-picrylhydrazyl radical by Oroki herbals, Swedish bitters and Yoyo bitters

Table 2: IC_{50} of Oroki herbal, Swedish bitters and Yoyo bitters on inhibition of TBARS, DPPH, HRBC stabilization, and anti-denaturation of BSA

Test samples	IC_{50}			
	TBARS assay (100-500 μl v/v%)	DPPH assay (25-500 μl v/v%)	Stabilization of HRBC assay (60-460 μl v/v%)	Anti-denaturation of BSA assay (20-100 μl v/v%)
Gallic acid	83.05	1.97		
Oroki herbal	187.8	2.32	31.14	352.11
Swedish bitters	274.3	3.37	2.62	181.82
Yoyo bitters	555.8	5.91	22.55	210
Diclofenac sodium			0.4	61.57

TBARS: Thiobarbituric acid reactive substances, DPPH: 1'1'-diphenyl-2-picrylhydrazyl, HRBC: Human red blood cell membrane, BSA: Bovine serum albumin, IC_{50}: Inhibitory concentration 50%

for DPPH scavenging activity of gallic acid, Swedish bitters, Yoyo bitters and Oroki herbal mixture were 1.97, 2.32, 3.37, and 5.91 μl v/v%, respectively [Table 2].

Data in Figure 4 exhibits that diclofenac sodium (2-[(2,6-dichlorophenyl)amino]benzeneacetic acid sodium salt), Swedish bitters, Yoyo bitters and Oroki herbal mixture stabilized HRBC membrane against hypotonicity-induced hemolysis in a concentration-dependent manner. The IC_{50} values for HRBC membrane stabilization against hypotonicity-induced hemolysis of diclofenac sodium, Swedish bitters, Yoyo bitters and Oroki herbal mixture were 0.4, 2.62, 22.55, and 31.14 μl v/v%, respectively [Table 2]. Furthermore, data in Figure 5 shows that the diclofenac sodium, Swedish bitters, Yoyo bitters and Oroki herbal mixture inhibited heat-induced protein denaturation in a reverse concentration-dependent manner; the IC_{50} values for this inhibition were 61.57, 181.82, 210.00, and 352.11 μ v/v%, respectively [Table 2].

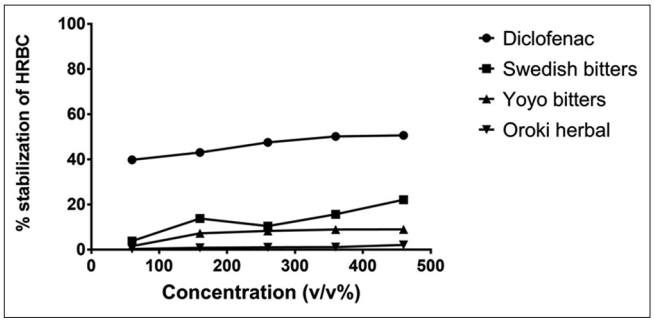

Figure 4: Percentage stabilization of hypotonicity-induced hemolysis of human red blood cell membrane by Oroki herbals, Swedish bitters and Yoyo bitters

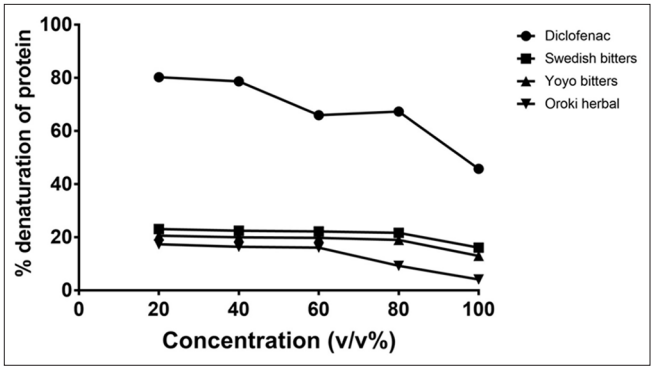

Figure 5: Percentage inhibition of thermal-induced protein denaturation by Oroki herbals, Swedish bitters and Yoyo bitters

DISCUSSION

The mechanism of tissue inflammation injury has been attributed, in part, to the release and propagation of oxidants as free radicals. These chemical radicals are important mediators in provoking or sustaining inflammatory processes, and consequently, their neutralization by phyto-antioxidants may attenuate inflammatory response [15].

In this study, the capacities of Oroki herbal, Swedish bitters and Yoyo bitters to act as antioxidants and anti-inflammatory agents were evaluated using *in vitro* models. Oroki herbal had the highest TAC followed by Yoyo bitters and Swedish bitters. This suggested the capacity of the studied polyherbal preparations to reduce molybdenum (VI) to molybdenum (V). This event could be attributed to the readiness of antioxidant phytochemicals present in them to donate a hydrogen ion to the reactive molybdenum (VI) [9]. The previous studies had shown the presence of polyphenols and flavonoids as antioxidants with the capacity to reduced molybdenum (VI) to molybdenum (V) [16,17]. Furthermore, Oroki herbal exhibited a higher capacity to neutralize the toxic end product of lipid peroxidation when compared with Swedish bitters and Yoyo bitters. Previous findings had also shown that plant extracts containing antioxidants could inhibit TBARS [18,19]. Free radical elicited membrane lipid peroxidation has been shown to be one of the events that occur during inflammation and oxidative stress mediated tissue damage [20,21].

Further investigations showed that Oroki herbal exhibited the highest capacity to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions and neutralize DPPH to $DPPHH^{+}$. This indicated that Oroki herbal might contain the most phenolic and flavonoid compounds with antioxidant activity when compared with Swedish bitters and Yoyo bitters. Other plant extracts have been shown to reduce ferric ion and donate H^{+} to DPPH as a mechanism of scavenging these unstable chemicals [22].

The evaluation of anti-inflammatory potentials of Oroki herbal, Yoyo bitters and Swedish bitters showed that Swedish bitters exhibited the highest stabilization effect against hypotonicity-induced hemolysis of HRBC membrane. This suggested that

Swedish bitters may contain the highest anti-inflammatory compounds. According to the previous study, both erythrocyte and lysosomal membrane have a similar molecular composition, hence, any agent that could stabilize the red blood cell membrane from hemolysis would provide a good insight into the anti-inflammatory mechanism of action [21]. More so, epidemiological and experimental studies have suggested an association between acute and chronic inflammation and risk of developing numerous pathological disorders [23,24]. Hence, natural compounds from plants that mitigate against inflammation could serve as therapy in the management of inflammation-mediated disorders. In addition, it could serve as a basis for the use of medicinal plants as traditional remedies against various inflammatory diseases [25].

In another study, Swedish bitters exhibited the highest effect against heat-induced denaturation of protein when compared with the other herbal preparations in a reverse concentration-dependent manner. Hence, this strengthens the previous observed claim in this study that Swedish bitters possess the highest anti-inflammatory property when compared with Oroki herbal and Yoyo bitters. The previous study had reported that the mechanism of protein denaturation probably involves modifications in the electrostatic, hydrogen, hydrophobic, and disulfide bonds of the protein structure. The reverse concentration-dependent response exhibited by the herbal preparations is in line with the statement made by Williams *et al.* [26] and Anyasor *et al.* [27] that the anti-denaturation properties of plant extracts are usually exhibited at low concentrations. Thus, data from this study had shown that Oroki herbal mixture exhibited the highest antioxidant effect while Swedish bitters had the highest anti-inflammatory effect.

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