Composition and in vitro Antioxidant Capacity of Yoyo Bitters

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Abstract: Bitters are beverages, often alcoholic that is flavoured with herbal essences that give it a bitter or bittersweet flavour. They are produced from root and herb extracts of plants and spices. Bitters are marketed as a “cure-all” patent medicine in Nigeria and a lot of them have never been subjected to scientific scrutiny. Hence, this study was aimed at ascertaining the composition and in vitro antioxidant capacity of one of such bitters, with the hope that this will help give some credence to its broad pharmacological claims. The results revealed that Yoyo bitters have high moisture content and small quantities of some essential food nutrients. The aqueous and ethanolic extracts of the bitters studied indicates that generally it contains in varying degrees amino acids, proteins, saponins, tannins, alkaloids, glycosides/reducing sugar, terpenoids and flavonoids. The results equally revealed that yoyo bitters contain significant amounts of Na, K, P, Ca, Mg, Zn, Mn, Fe and Cu, while Pb, Cr and Se were not detected. The antioxidant scavenging effects of bitters revealed that yoyo bitters have IC50 values for 50% OH radical inhibition of 278.95 mg/ml indicating that the bitters can be said to have a relatively high antioxidant capacity as claimed by the producers of the bitters.

Key words: Yoyo bitters • Antioxidant • Medicinal plants and pharmacological properties

INTRODUCTION

The term “bitters” as it is used presently, is a beverage, often alcoholic, flavoured with herbal essences that gives it a bitter or bittersweet flavour. The generic term applies to all bitter liquors and herbal bitters. Bitters are produced from herb and root extracts, from the narcotic components of (primarily) tropical and subtropical plants and spices. They are usually dark in colour and valued for their ability to promote appetite and digestion hence their use as patent medicine and as a aid in digestion and flavouring in cocktails. Bitters are made up of numerous groups of chemical compounds extracted from the herbs and roots (medicinal plants) that have the common characteristic of a bitter taste and act to increase the vital energy centres in the body [1, 2].

Unorthodox traditional medicine practice which employs the use of herbs (medicinal plants), have in recent times been gaining much publicity and recognition, for their solution to ailments seemingly elusive to the system of orthodox medical practice. Medicinal plants have been defined as those plants which contain in one or more of their organs, substances that can be used for the synthesis of useful drugs. Medicinal plants have been defined and associated with gods and spiritual invocations. However, with the advent of science, civilisation and Christianity, there has been a drastic decline in this practice. Modern pharmacology had its origin in these medicinal plants and till date some drugs are products of active components from plants [3, 4]. The differences in terms of medication between orthodox and unorthodox/traditional medicine seems to be closing fast as the current trend is that they are both adopting practices from each other [3, 4]. This has led to the resurgence of an ancient remedy for digestive problems in the repackaging of “herbal bitters” and products like it in an “orthodox way”.

Bitters have been claimed to help heal piles/haemorrhoids and improve sexual function. It equally leads to enhanced blood circulation, purification of blood by the kidneys, blood pressure regulation through arterial dilatation and prevents formation of
kidney stones and cleanses the colon of impurities. Bitters have also been said to possess anti-tumour especially against colo-rectal cancers. They are also said to have anti-inflammatory, antibiotic and antifungal properties.

Bitters have also been said to ensure good digestion of fats and oils and proper functioning of the liver, reduce accumulated fat (triglycerides) and cholesterol levels thereby conferring on it hypolipidaemic properties. They are said to reduce excess body fat and promote healthy weight loss, act as a liver tonic and body detoxifier; being hepatoprotective and enhancing its functions generally and helping in body detoxification.

In modern herbal medicine, “bitter principles” occupy a central place in herbal therapeutics, bearing the acrid constituents. Most people consuming herbal medicines complain about the bitterness of the medicines prescribed. This is the only defining attribute of herbal medicine and the only feature to set it apart from other therapies [2, 4]. In the past, our traditional diets were not devoid of bitter foods as is presently the case in most modern diets, hence Green [5] desires that we see the medicinal side of bitters in an entirely different light in other that we use it to prevent what he termed the “Bitter Deficiency Syndrome” of our era, which in his opinion is the predisposing factor to many ailments of our time [2, 5]. All these make the study of the constituents and pharmacological effects of present day bitters desirable.

MATERIALS AND METHODS

Material: Yoyo bitters were purchased from reputable pharmaceutical stores opposite the University of Benin Teaching Hospital (UBTH), Ugbowo Lagos Road, Benin City, Edo State, Nigeria. The bitters were bought as liquid formulations and stored at room temperature throughout the period of the experiment.

Proximate Analysis: The moisture content of the herbal bitters was determined using the gravimetric method [6]. The ash content was estimated using the method of AOAC [7]. The fat content was determined using the method of AOAC [8]. The crude fibre content was determined by the difference in weight after calcination, following the digestion of the sample in sulphuric acid and sodium hydroxide solutions and the residue being calcined [9]. The protein content of the bitters was determined from the organic nitrogen content by Kjeldahl method [9]. The carbohydrate content of the bitters was determined by the difference method by adding moisture, fat, protein and ash content and the value deducted from 100 [9]. The reducing sugar content of the bitters was determined using the dinitrosalicylic acid method [10]. The glucose content was determined using the glucose oxidase method [11]. Triplicate measurements were performed and the mean computed.

Alcohol Composition Analysis: The determination of the alcohol content was done using the AOAC [7] method.

Extraction of Bitters for Qualitative Phytochemical Analysis: because there may be differential solubility in the constituent of the bitters relative to the polarity of the solvent used the bitters were further extracted in distilled water and ethanol. Aqueous Extraction: 10ml of bitters sample was added to 90ml distilled water and boiled on slow heat for 2hours. It was then filtered using a Whatmann No. 42 filter paper (125mm); the filtrate was collected and further concentrated to make the final volume one-fourth of the original volume and stored at 4oC in an air tight container [12]. Preparation of ethanolic extract: 10ml of the bitters sample was taken and put into 50ml of absolute ethanol in a flat bottom flask. The flask was plugged with cotton wool and then kept on a rotary shaker at 190-220rpm for 24hrs. After 24 hours the sample-solvent mix was then filtered using a Whatmann No. 42 filter paper (125mm); the filtrate was collected and the solvent evaporated to make the final volume one-fourth of the original volume and stored at 4oC in an air tight container [12].

Qualitative Phytochemical Analysis: The test for phytosterol/steroids, amino acids, protein, saponins, tannins, alkaloid, cardiac glycoside, terpenoid, flavonoids, phlobatannins, was determined using the method described in Santhi and colleagues [12]. The test for glycosides/reducing sugars was carried out using the method described by Onyeike and Osuji [9]. The Borntrager’s test was used for the detection of anthraquinones [13].

Quantitative Phytochemical Analysis: The determination of total phenols was by spectrophotometric method [14], while alkaloids was as described by Harborne [15] and Edeoga and colleagues [14]. The determination of tannin was by the Van – Burden and Robinson method [14], while the determination of saponin was as described by Edeoga and colleagues [14]. The determination of flavonoids was by the method of Boham and Kocipai-Abyazan [16], while that of cyanogenic glycosides was by the AOAC [7] method. Elemental Analysis: Extraction and
determination of calcium and magnesium in the bitters sample were determined by the titrimetric method using ethylenediaminetetraacetic acid (EDTA), [7]. The sodium and potassium concentrations were determined using the flame photometer (JENWAY PFP 7 model) [7], while phosphorus was determined by Olsen and Sommers [17] and the AOAC [7] methods. The determination of the trace metals iron, zinc, manganese, copper, lead, chromium and selenium were done using the bulk scientific VGP210 atomic absorption spectroscopy/spectrophotometry, [7].

**Determination of the Total Antioxidant Capacity:** The ability of the herbal bitters to scavenge 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) radical cation (ABTS+) which gives an idea of the total antioxidant capacity, was determined by the improved spectrophotometric version described by Re and colleagues [18].

**ABTS Radical Cation Decolourisation Assay:** In this improved version, ABTS+--the oxidant, will be pregenerated by persulfate oxidation of 2,2',-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) – (ABTS2-). Triplicate measurements were performed and the mean computed. DPPH free radical scavenging activity: DPPH (2,2-diphenyl-1-picrylhydrazyl radical) scavenging activity of the herbal bitters was measured by the spectrophotometric method described by Jain and colleagues [19]. Triplicate measurements were performed and the mean computed. Hydroxyl radical scavenging activity: This was assayed as described by Kunchandy and Rao [20] with a slight modification [21]. The assay is based on quantification of the degradation product of 2-deoxyribose by condensation with TBA. Triplicate measurements were performed and the mean computed. Statistical analysis: The results are expressed in Mean±SEM. Students t-test was used to compare the means. P<0.05 was considered significant.

**RESULTS**

Table 1 shows that Yoyo bitters has a high moisture and alcohol content, no fibre and a higher ash content compared to other food nutrients.

Table 2 shows that considering both the aqueous and ethanolic extracts of the bitters, the bitters used in this research contains amino acids, proteins, saponins, tannins, alkaloids, phlobatannins, plant sterols (steroids), terpenoids and flavonoids but did not contain cardiac glycosides, glycosides/reducing sugar and anthraquinones. As the key indicates, these phytochemicals were present in varying degrees of slight, moderate and high. The quantitative phytochemical composition of the bitters, indicate saponins as the highest constituent and tannins the least among those determined.

### Table 1: Proximate and Alcohol composition of the Yoyo Bitters

<table>
<thead>
<tr>
<th>Quantity in Yoyo Bitters (%)</th>
<th>Ash</th>
<th>Moisture</th>
<th>Fibre</th>
<th>Fat &amp; oil</th>
<th>Protein</th>
<th>Carbohydrates</th>
<th>Glucose</th>
<th>Reducing sugar</th>
<th>Alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.75±0.03</td>
<td>92.27±1.12</td>
<td>0.00±0.00</td>
<td>0.68±0.05</td>
<td>0.88±0.02</td>
<td>2.42±0.07</td>
<td>0.61±0.01</td>
<td>0.99±0.03</td>
<td>10.51±0.05</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Qualitative and Quantitative Phytochemical Composition of Yoyo Bitters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
<th>Quantitative composition of yoyo bitters (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytosterol</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Amino acids</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>++</td>
<td>6.43±0.23</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>0.61±0.01</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Glycosides/reducing sugar</td>
<td>-</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>ND</td>
<td>ND</td>
<td>0.15±0.00</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
<td>+++</td>
<td>2.15±0.06</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>+++</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td></td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Total phenols</td>
<td>ND</td>
<td>ND</td>
<td>1.46±0.05</td>
</tr>
</tbody>
</table>

Key: +++ = Highly Present; ++ = Moderately Present; + = Slightly Present; = Absent; ND = Not Determined
Table 3: Quantitative Mineral Composition of the Yoyo Bitters

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>K</th>
<th>P</th>
<th>Fe</th>
<th>Zn</th>
<th>Mn</th>
<th>Cu</th>
<th>Pb</th>
<th>Cr</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantity in yoyo bitters (mg/100ml)</td>
<td>20.20±0.78</td>
<td>243.05±2.34</td>
<td>5.80±0.38</td>
<td>165.00±7.64</td>
<td>27.09±1.59</td>
<td>7.50±0.17</td>
<td>3.00±0.12</td>
<td>0.85±0.04</td>
<td>2.00±0.15</td>
<td>&lt;DL</td>
<td>&lt;DL</td>
<td>&lt;DL</td>
</tr>
</tbody>
</table>

Where <DL means less than detection limit. Triplicate measurements were performed and the mean computed. The values are expressed as Mean±SEM.

Table 4: Antioxidant Capacity by IC₅₀ Values of the Control and Test Bitters using various methods

<table>
<thead>
<tr>
<th>Groups</th>
<th>IC₅₀ (mg/ml) by Total antioxidant capacity</th>
<th>IC₅₀ (mg/ml) by DPPH Scavenging ability</th>
<th>IC₅₀ (mg/ml) by Hydroxyl Radical scavenging ability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>0.08±0.00</td>
<td>0.05±0.01</td>
<td>204.64±4.68</td>
</tr>
<tr>
<td>Yoyo bitters</td>
<td>1.71±0.00</td>
<td>26.35±1.26</td>
<td>278.95±15.22</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM. Values in the same column with different superscript letters differ statistically significantly (P<0.05) from one another.

*Standard for total antioxidant capacity and DPPH scavenging ability was ascorbic acid while that of hydroxyl radical scavenging ability was mannitol.

Table 3 summarizes the means of the quantitative elemental composition of the bitters, with magnesium being the highest constituent and manganese the lowest. The results show that the bitters contain significant amounts of Na, K, P, Ca, Mg, Zn, Mn, Fe and Cu, while Pb, Cr and Se where not detected.

Table 4 shows that Yoyo bitters has the ability to inhibit the ABTS radical, scavenge DPPH and the hydroxyl radical, hence it has antioxidant capacity. Statistical evaluation however shows that the concentration required for 50% inhibition of ABTS and DPPH by ascorbic acid (IC₅₀) and 50% inhibition of the OH radical by mannitol (IC₅₀) is significantly (P<0.05) lower than that of Yoyo bitters.

DISCUSSION AND CONCLUSION

Discussion: Though herbal bitters are presently being marketed as a “cure-all”- patent medicine in Nigeria, a lot of them have never been subjected to scientific scrutiny. Hence this study was aimed at ascertaining the composition and in vitro antioxidant capacity of one of such bitters, with the hope that this will help give some credence to its broad pharmacological claims. The results of table 1 showed that the proximate composition of Yoyo bitters has high moisture content and small quantities of some essential food nutrients. The results of this research is in agreement with the findings of Awa, et al., (2013) [22], who worked on Swedish bitters and found that they contained carbohydrates, proteins, lipids, ash and a high moisture content, though with some variance in terms of the amount of these constituents compared to the amount of the same constituents found in the Yoyo bitters of this study. Proteins, fats and carbohydrates are essential for life and studies have indicated that life is sustained by nutrient mixtures in which every component is definable chemically and soluble in water [23]. The aqueous and ethanolic extracts of the bitters studied indicates that generally it contains in varying degrees amino acids, proteins, saponins, tannins, alkaloids, glycosides/reducing sugar, terpenoids and flavonoids. The locally produced Yoyo bitters were found to be moderate in its content of saponins and flavonoids as these were its highest constituents. Bitters are made up of numerous groups of chemical compounds extracted from the herbs and roots, they have the common characteristic of a bitter taste. The chemical compounds that confer this bitter taste have been classified into those that are glycosides, alkaloids, terpenoids (especially the diterpenes, triterpenes and sesquiterpenes) and those that are flavonoids and tannins [24]. Table 2 indicates that the bitters of this study contain the major classes of substances that rightly confer on them the name “bitters” [25-27]. The qualitative and quantitative estimation of the phytochemical constituents of a medicinal plant is considered to be an important step in medicinal plant research [28]. The presence of these secondary metabolites in plants probably explains the various uses of plants for traditional medicine [29]. Herbal bitters are claimed to have so many medicinal properties. In relation to the known medicinal uses of the identified phytochemical constituents of the herbal bitters (Table 2) and depending on the medical needs/ailment of the patient, yoyo bitters can easily be prescribed if it has the constituent that will help improve the ailment. Saponins, flavonoids and total phenols are in significant quantity and relatively higher than other phytochemicals quantitatively determined and this may be associated with the antioxidant properties of yoyo bitters. Alkaloids and their derivatives have been certified to have important biological, physiological and medicinal effects in man.

Tannins as a group of compounds have received a great deal of attention in recent years, since it was suggested that the consumption of tannin-containing beverages, especially green teas and red wines, can cure or prevent a variety of ills [30]. Many human physiological activities, such as stimulation of phagocytic cells, host -mediated tumour activity and a wide range of
anti-infective actions, have been assigned to tannins [31-36]. Some of these same claims associated with yoyo bitters may be traced to its tannin content. Flavonoids are regarded as having anti-inflammatory (inhibit inflammatory metabolites and granulation tissue formation), anti-allergic (inhibit histamine release) and anti-oxidant effects. They are well known for their therapeutic function through an ability to inhibit enzyme system (e.g., lipooxygenase, cyclo-oxygenase, elastase and aldose reductase) as well as free radical scavenging and co-factor activity for the anti-oxidant, vitamin C [37, 38]. Other actions demonstrated by different flavonoids include hepatoprotective, anti-spasmodic, hypocholesterolaemic, diuretic, anti-viral and anti-bacterial effects [39, 40]. Some of these same claims associated with yoyo bitters may be traced to its flavonoid content.

Phenols and phenolic compounds may be used as anti-microbial agents as they have antibacterial and antifungal properties [41, 42]. They are also said to be antioxidant, immune enhancers, anti-clotting and hormone modulators [41, 42]. Some of these same claims associated with yoyo bitters may also be traced to their phenol content.

Saponins when present in high concentration in the plants as compared to other phytochemicals, will likely indicate that extracts from these plants are used in wound healing and bleeding treatment [42, 43]. Saponins have properties of precipitating and coagulating red blood cells and they also have cholesterol binding properties (hence their hypolipidaemic/hypocholesterolaemic property), formation of foams in aqueous solutions and haemolytic activity [42, 43]. Some of these same claims associated with yoyo bitters may therefore be traced to their saponin content.

The results of this analysis show that the cyanogenic glycoside content of yoyo bitters (0.18%) is safe and too low to cause any toxicity in the prescribed dosage the bitters are normally consumed; instead the range is enough to harness any positive effect known in the medicinal use of cyanogenic glycosides.

Evidence suggests that cyanogenic glycosides may have beneficial effects in animals that consume them. These compounds do have anticarcinogenic activity invitro according to recent research [44-47]. Cyanogenic glycosides have also been said to have sedative and expectorant effect in the respiratory tract [44-47]. They have also been said to have a cooling effect as hydrocyanic acid cuts down heat at a cellular level. Examples include- peach, apple and pear which are all cooling fruits that contain cyanogens [44-47]. Most of the claims of yoyo bitters may therefore be attributed to its phytochemical constituent.

Table 3 shows that yoyo bitters contain significant amounts of Na, K, P, Ca, Mg, Zn, Mn, Fe and Cu, while Pb, Cr and Se were not detected. Recent promotion of herbs as health foods commonly includes reference to their mineral contents [48]. So yoyo bitters can be of help in mineral deficiencies especially in cases of Na, K, P, Ca, Mg, Zn, Mn, Fe and Cu deficiencies. Sodium (Na) and Potassium (K) play significant roles in acid-base balance, fluid balance, nerve function and proper neuromuscular and cardiac activity/function [49]. Phosphates contribute to overall health by its involvement in energy transfer, phosphorylation/dephosphorylation reactions, lipid metabolism and acid-base balance and enzyme action [49]. Normal extracellular calcium concentrations are necessary for blood coagulation and for the integrity of intracellular cement substances and integrity and proper functioning of nerves, skeletal muscle and heart/smooth muscle [50]. The presence of zinc in yoyo bitters could mean that they can play valuable roles in the management of diabetes, which result from insulin malfunction [50, 51] as well as explain the claim that the bitters play a role in free radical scavenging activity, improved growth, sexual activity and wound healing [49, 50]. The fact that yoyo bitters have IC50 values for 50% OH radical inhibition of 278.95 mg/ml indicates that the bitters can be said to have a relatively high antioxidant capacity as claimed by the producers of the bitters. Herbal products have been said to have phytochemical constituents that confer on them antioxidant properties [52, 53], this is in agreement with our findings as the fact that these bitters can inhibit the OH and DPPH radical confirms the antioxidant capacity of the herbal bitters. Antioxidant constituents can delay or inhibit the oxidation of lipids and other compounds by inhibiting the propagation of oxidation chain reaction [53, 54]. Primarily, antioxidant effect is due to phenolic compounds such as phenolic acid, flavonoids and phenolic diterpenes and their mode of action as antioxidant compounds is due to their redox reaction properties which can absorb and neutralize free radicals by quenching singlet and triplet oxygen [53, 55].

**CONCLUSION**

The biochemical assay results of this study with inferences derived from some of the already established effects of some of the phytochemical and mineral
constituents of these bitters, gave some evidence that the herbal bitters of this study may be said to have the potential or possibility of having the following pharmacological properties - hypolipidaemic/hypocholesterolaemic, hypoglycaemic, anti-anaemic and anti-inflammatory, stimulant and immunity-boosting/immuno-modulatory, choleretic/hepatoprotective and antihepatotoxic, invitro and in vivo antioxidant capacity and by extension anticarcinogenic/antineoplastic/antimutagenic/antitumour as well as diuretic/vasodilatory and antihypertensive properties and the ability to protect against/prevent coronary artery disease and cardiovascular diseases generally.

REFERENCES


