Quantitative Analysis of Chemical Contaminants in Ghanaian Herbal Alcoholic Bitters

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Abstract

Ten popular Ghanaian herbal alcoholic bitters were selected for the investigation of organic and inorganic chemical contaminants using a combination of LR/EI-GCMS, HR/ESI-LCMS, ICP-AES and AAS techniques. The ability of these drinks to inhibit microbial growth and the effects of shelf-life on the physicochemical parameter pH were also studied. The results showed that Ghanaian herbal alcoholic bitters have a complex matrix of various compounds which are difficult to isolate. Di-n-octyl phthalate (DOP) was present in all the ten herbal alcoholic bitters studied but the amounts of DOP isolated from the five drinks studied into detail were 7.84mg/L for MD, 12.53mg/L for KA, 30.72mg/L for HA, 36.22mg/L for AG and 15.76mg/L for OG. The level of DOP in all the samples exceeded by a large amount the WHO limit of 8µg/L. The herbal alcoholic bitters, as packaged did not inhibit the growth of pathogenic bacteria strains Staphylococcus aureus and Escherichia coli. With the rich organic load per bottle and the fact that these drinks were found to be generally acidic, microbial invasion and growth is highly possible. The potential to further complicate the chemistry of these products when microbes metabolize the complex organic compounds in the drinks can lead to microbial toxicity in addition to direct pathogenic effects. The ten Ghanaian herbal alcoholic drinks analyzed generally contained various metals at different levels compared to the WHO permissible limits. The levels of the trace metals Al and Fe in all the herbal alcoholic drinks exceeded the WHO recommended daily intake (RDI) of 0.2mg/L and 15mg/L respectively. The levels of As exceeded the WHO...
RDI of 0.01mg/L in all the drinks except in PB, OG and CL. However, levels of Hg, Cd, Se and Pb were below detectable limits for all the drinks studied. We hope that this data will be applied to the continued study of this problem in more depth and to allow this to inform a policy process that will lead to greater quality assurance of these drinks and safeguard the health of Ghanaian consumers.

Keywords: Heavy metals, Phthalates, LC-MS, GC-MS, ICP-AES, AAS

Introduction
Ghana like most countries in Africa has a low life expectancy of 60 years and below [1, 2]. Low life expectancy in many African countries is direct consequence of civil war, hunger, poverty, HIV/AIDS; amongst other things [1, 2]. Some of the factors that affect life expectancy are very obvious whilst others are hidden or silent. For a chemist, one factor that affects average life expectancy stands out and this is the exact chemical composition of what people eat, drink and apply to their bodies. The exact chemical components of most of the products that Ghanaians use are not known. Most of these products do not come in proper packaging and if they do the labels give little information about the chemical composition. There is a need for people in Ghana and other West Africans to conduct scientific research that will provide detailed chemical knowledge of everything they eat, drink, apply to their bodies or use as medicines. In cases where items are packaged well, it is important for the public to realize that it is not the beauty of the packaging that matters but, the information it provides on the labels. As far as herbal medicinal products are concerned, the use of "Chemical Constituent Labels" for the major active ingredients in the products must be encouraged in Ghana.

The importance of herbal medicinal products and its increasing use by Ghanaians and in other parts of the world cannot be over-emphasized. However, the rate at which herb-based products are flooding the Ghanaian market without regulation is a big worry for health experts and other stakeholders. Generally, herbal medicines are believed to be benign and not to cause severe toxicity or have any side effects [3, 4]. This belief coupled with lower costs as compared with conventional medications is a major reason for the wide patronage of herbal medicinal products in Ghana [3, 5]. However, the use of herbal medicines can cause severe toxicity or complications and even death [4]. These effects are due to some potentially toxic constituents and contaminants they contain if there is no measure in place to prevent their inclusion right from the point of collection of materials, production, packaging and storage. The potential causes of toxicity of herbal products includes; the use of inherently toxic herbs mainly as a result of misidentification of the herbs, variability in active or toxic ingredients due to growing conditions, processing or preparation and the inevitable mechanisms of adulteration and contamination [4].

Furthermore, Ghanaian physicians’ knowledge of potential herbal medicine toxicities is limited because their training has little to do with herbs and herbal toxicities, but more with conventional medicine. Familiarity of doctors with potential
toxicities of herbal medicine is therefore very limited and thus, whenever they emerge as medical emergencies, it becomes difficult to readily identify and treat these conditions [5].

In Ghana, one interesting area for research is that of alcoholic herbal bitters. These drinks are aqueous extracts of selected plants or herbs combinations to which some quantities of alcohol (18-45% Volume) are added.

Figure 1: Ghanaian alcoholic bitters packaged in various forms with plastic or glass bottles

Currently, there is a proliferation of alcoholic herbal bitters on the Ghanaian market, with increased sophistication in their packaging and a large number of advertisements in the media. Alongside the belief of utmost safety these make the products appeal to a huge section of the population including both genders, young or old. From the perspective of the Ghanaian consumer, there are many reasons for the wide patronage of these alcoholic drinks and these include health, recreation and vitality or even fertility. While the safety and quality of medicinal plant materials and finished herbal medicinal products have become a major concern globally, for authorities, pharmaceutical industries and a section of the public [4, 5], Ghanaian herbal alcoholic bitters on the contrary have no information available to the public on the various plants used for the formulation of these drinks. Directions of use, quantities to be consumed on a daily basis, the rate or frequency of consumption etc are all left to the discretion of the consumer who has no idea of the plant parts used for the formulation of these alcoholic drinks.

Hence, there have been several reports in the Ghanaian media of incidents of priapism, dysuria, strangury and even death attributed to the consumption of herbal alcoholic bitters by the victims [6]. The production of these drinks is widespread and poorly regulated and several popular brands are produced in what can best be described as a bathroom.
In this study, ten popular Ghanaian herbal alcoholic bitters were selected and investigated for the presence of organic and heavy metal contaminants alongside the measurement of physicochemical parameter pH. Organic compounds present in the drinks were analyzed by high resolution electrospray ionization liquid chromatography mass spectrometry (HR-ESI/LCMS), low resolution electron impact gas chromatography mass spectrometry (LR-EI/GCMS) and nuclear magnetic resonance spectroscopy (NMR). Heavy metal contaminants in the drinks were analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES) and atomic absorption spectrometry (AAS).

Since all the selected drinks did not have manufacture and expiry dates on the bottles, the ability of these drinks to inhibit the growth of Staphylococcus aureus NCIMB12702 and Escherichia coli NCIMB12210 were also evaluated using ampicillin, oxolinic acid and oxytetracycline as positive controls.

**Materials and Methods**

**Chemicals, standards and reagents**

Hexane, methanol, formic acid, ethyl acetate, dichloromethane, acetonitrile and tetrahydrofuran were purchased from Sigma-Aldrich, UK Ltd. nitric acid (65% suprapur HNO$_3$), hydrochloric acid 35% suprapur HCl, and perchloric acid (70% suprapur HClO$_4$) were obtained from E. Merck, Darmstadt, Germany. Analytical reference standards for heavy metals were also purchased from Sigma-Aldrich, UK Ltd.

All other chemicals and reagents used were of analytical grade and obtained from standard suppliers.

**Containers and cleaning process**

New Pyrex glass conical flasks, separating funnels and Teflon tubes were used. These containers were rinsed with de-ionized (DI) water and immersed in a warm liquid soap bath for two days, then rinsed with DI water, immersed in 10% HNO$_3$ at room temperature for three days. The containers were again rinsed three times with DI-water, and then immersed in 50% HNO$_3$ bath at 90ºC for 24hours. They were further rinsed with DI-water several times and placed in a clean oven at 60ºC overnight. The containers were removed from the oven and allowed to cool-down and double bagged in re-sealable new polyethylene bags and stored at room temperature until use.

**Sample Collection**

Ten Ghanaian herbal alcoholic gin bitters were purchased directly from the manufacturers at their premises in Accra. The bitters included: Alomo (KA), Opeimu (OP), Cocoa Liqueur (CL), and Ogidigidi (OG) (from Kasapreko Company Ltd); Herb Afrik (HA) and Mandingo (MD) (from GIHOC Distilleries); Agya Appiah (AG), (from Agya Appiah Bitters Ltd); and Pusher (PU), Playboy (PB) and Cargo (CG), (from Baron's Distilleries). Two different batches of product samples were purchased in the months of May and November, 2012 respectively. Each box of the herbal alcoholic drink purchased contained six 750ml bottles of the drinks. The six bottles of each product in the same box were treated as the same batch samples.
allowing enough volumes for all the desired analysis to be performed for each batch.

All the ten selected drinks were studied for trace metals content and anti-microbial activity. Additionally, five (5) most supposedly patronized brands out of the ten (Alomo, Mandingo, Herb Afrik, Agya Appiah and Ogidigidi) were chosen to investigate for any isolable organic compounds besides, the plant phytochemicals.

**Measurement of pH of herbal alcoholic bitters**

All samples purchased in May and November 2012 were brought to a common Room Temperature (Laboratory condition) for one week before any sort of analysis was carried out. The pH of all the different brands were measured at day 0 under three different set of conditions (4°C, room temperature and 37°C) and the measurement of pH repeated at two weeks intervals for a total of ten weeks. All pH measurements were conducted using a Mettler Toledo FE20/EL20 pH meter.

**Analysis of Organic Contaminants in Herbal Alcoholic bitters**

The selected five Ghanaian herbal bitters KA, HA, MD, AG and OG were partitioned in high purity (HPLC) grade dichloromethane (DCM) by slight modification of Kupchan’s method [7]. A fresh bottle of each herbal bitters containing about 750ml of the alcoholic extract was poured into a 2L separating funnel and phase adjusted with about a 100ml of high purity (HPLC) grade methanol [7]. Each herbal drink sample was extracted with 0.85L DCM three times (3 x 0.85L). The procedure was run in duplicates for each class of herbal bitters. The combined extract obtained for each was then rotary evaporated to obtain a light yellow liquid. Flash column chromatography was then conducted on silica gel 200-425 mesh (hexane/ethylacetate-7:3) and TLC was employed to monitor column fractions and purity of compounds using pre-coated silica gel for each drink. Compounds were detected by examination under ultraviolet light (λ 250nm) or using an iodine chamber. Pure compounds of phthalates isolated were then characterized by mass spectrometry and NMR spectroscopy. High resolution mass spectra (HR/LC-MS) were measured on ESI-MS (LTQ XL/LTQ Orbitrap discovery) instrument. Additionally, low resolution/gas chromatography-mass spectrometry (LR/GC-MS) and high resolution/high performance liquid chromatography-mass spectrometry (HR-LC-MS) were used to verify the structures of the phthalates isolated.

**Extraction of phthalates from Sintex tank material**

The synthetic material used to mold Sintex or Polytanks comes in the form of thin coiled wires. A sample of this material was obtained from one of the manufacturing companies of these tanks, in Accra.

The material was chopped into tiny pieces and soaked in a H₂O:CH₃CH₂OH (1:1) mixture for one week. The mixture was then decanted and extracted with an equal volume of 100% DCM. Likewise, samples of PVC pipes were obtained from Interplast and Duraplast, Accra, Ghana. The pipes were shredded and 3.0g of each was soaked in H₂O:CH₃CH₂OH (1:1) and extracted as described above. The extracted samples were subjected to low resolution gas chromatography mass spectrometry (LR/GC-MS).
Determination of the antibacterial activity of Ghanaian herbal alcoholic bitters
The antibacterial activity of all the ten Ghanaian aqueous alcoholic drinks were evaluated against Gram positive strain; Staphylococcus aureus NCIMB12702 and Gram negative strain; Escherichia coli NCIMB12210 using the agar well-diffusion method [8]. Filter paper discs containing ampicillin (10µg), oxolinic acid (2µg) or oxytetracycline (30µg) were used as positive controls. In this procedure, strains were grown in Müller-Hinton (MH) broth to early stationary phase. Müller-Hinton agar plates were prepared and seeded with the test strain. Wells having the same diameter as the discs impregnated with antibiotic standards were created in the surface of the agar. The herbal alcoholic drinks were then diluted to between 10-50% of their volume with deionized water. Aliquots of 50μL of the undiluted as well as the various diluted samples were placed into each well. Control wells, each containing 50μL of 20% ethanol were also constructed. Each sample was tested in triplicates and at different serial dilutions of their original volumes. The plates were then incubated at
37°C for 24 hours after which zones of inhibition were measured as an indicator for bacterial susceptibility to the drink samples [8].

**Analysis of heavy metals in alcoholic bitters**
The heavy metal content of the ten brands of herbal alcoholic bitters was studied. About 5g of each sample was weighed into labeled 100ml polytetrafluoroethylene (PTFE) Teflon bombs. Ten milliliters of a concentrated mixture HNO₃:HCl:HClO₄ (1:3:0.5) was added to each sample and allowed to cool for 1 hour before gently heated till a colourless solution was obtained. The samples were then allowed to cool and diluted with de-ionised (DI) water to 50ml volume and filtered into cleaned high density polyethylene (HDPE) containers, analyzed and quantified by inductively coupled plasma atomic emission spectroscopy (ICP-AES) and atomic absorption spectrometry (AAS) for the detection of mercury (Hg).

**Quality Assurance/Quality Control**
Quality control (QC) samples including replicates and blanks were included in each batch of samples for analysis. Analysis of all blank samples showed no inherent bias in the method of analysis for the trace elements. All differences measured in concentrations between replicate pairs were well within the precision of the method for all trace elements. The accuracy of the method was checked by recovery assays with known amounts of analyte added to three herbal drink samples chosen and analyzed. The recovery values obtained for spiked samples were 99.0 ± 0.2% in CL, 93 ± 1.0% in KA, and 98 ± 0.5% in PU drinks respectively.

**Results**

**Table 1:** Summary of product information on each drink obtained by inspection

<table>
<thead>
<tr>
<th>Product</th>
<th>Total Volume (ml)</th>
<th>Alcohol content (%)</th>
<th>GFDB CERT</th>
<th>GSB CERT</th>
<th>Bottle type</th>
<th>Company address</th>
<th>Ingredients list</th>
<th>DATE OF Manufacture</th>
<th>DATE OF Expiry</th>
</tr>
</thead>
<tbody>
<tr>
<td>KA</td>
<td>750</td>
<td>42</td>
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<td>Glass</td>
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<td>NONE</td>
</tr>
<tr>
<td>HA</td>
<td>750</td>
<td>40</td>
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<td>YES</td>
<td>Glass</td>
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<td>NONE</td>
<td>NONE</td>
<td>NONE</td>
</tr>
<tr>
<td>MD</td>
<td>700</td>
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<td>Glass</td>
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<td>NONE</td>
<td>NONE</td>
<td>NONE</td>
</tr>
<tr>
<td>AG</td>
<td>750</td>
<td>40</td>
<td>YES</td>
<td>NO</td>
<td>Plastic</td>
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<td>NONE</td>
<td>NONE</td>
<td>NONE</td>
</tr>
<tr>
<td>OP</td>
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<td>Glass</td>
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<td>NONE</td>
<td>NONE</td>
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<td>NONE</td>
<td>NONE</td>
</tr>
<tr>
<td>CG</td>
<td>750</td>
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<td>NO</td>
<td>Plastic</td>
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<td>NONE</td>
<td>NONE</td>
<td>NONE</td>
</tr>
<tr>
<td>OG</td>
<td>750</td>
<td>38</td>
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<td>YES</td>
<td>Plastic</td>
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<td>NONE</td>
<td>NONE</td>
<td>NONE</td>
</tr>
<tr>
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<td>750</td>
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<td>YES</td>
<td>Plastic</td>
<td>YES</td>
<td>NONE</td>
<td>NONE</td>
<td>NONE</td>
</tr>
</tbody>
</table>

Key: GFDB: Ghana Food and Drug Board GSB: Ghana Standards Board CERT: Certification
Table 2: Variation of pH with increasing storage times under different conditions for KA

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Length of storage/pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 weeks</td>
</tr>
<tr>
<td>4</td>
<td>1.19</td>
</tr>
<tr>
<td>Room temperature (~26°C)</td>
<td>1.19</td>
</tr>
<tr>
<td>37</td>
<td>1.19</td>
</tr>
</tbody>
</table>

Figure 4: LR/EI-GCMS of DOP isolated from Alomo Bitters

Figure 5: Proposed fragmentation pattern of DOP in HR/ESI-LCMS (Also applicable to data obtained by LR/EI-GCMS)
**Figure 6**: LR/EI-GCMS profile of synthetic material used in the manufacture of Sintex tanks

**Figure 7**: HR/ESI-LCMS spectra of raw HA from two different batches overlaid for comparison
Figure 8: Mass average over the entire chromatograms for two different batches of HA overlaid for comparison (Figure 7)

Table 3: Concentration of heavy metals (mg/L) in Ghanaian Herbal Alcoholic Bitters

<table>
<thead>
<tr>
<th></th>
<th>Al</th>
<th>Cr</th>
<th>Cu</th>
<th>Fe</th>
<th>Mn</th>
<th>Ni</th>
<th>Zn</th>
<th>Se</th>
<th>Pb</th>
<th>As</th>
<th>Cd</th>
<th>Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KA</td>
<td>67.94±3.77</td>
<td>&lt;0.001</td>
<td>&lt;0.003</td>
<td>60.00±1.23</td>
<td>6.16±1.32</td>
<td>&lt;0.001</td>
<td>3.86±0.17</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>89.80±0.02</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>2</td>
<td>OP</td>
<td>43.74±1.87</td>
<td>&lt;0.001</td>
<td>&lt;0.003</td>
<td>43.43±5.82</td>
<td>2.44±0.47</td>
<td>&lt;0.001</td>
<td>5.64±0.19</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>92.90±0.02</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>3</td>
<td>HA</td>
<td>62.58±5.11</td>
<td>&lt;0.001</td>
<td>&lt;0.003</td>
<td>91.09±0.78</td>
<td>3.20±0.87</td>
<td>&lt;0.001</td>
<td>2.14±0.66</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>28.75±0.21</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>4</td>
<td>AA</td>
<td>72.22±7.35</td>
<td>&lt;0.001</td>
<td>&lt;0.003</td>
<td>46.83±2.25</td>
<td>2.20±0.13</td>
<td>&lt;0.001</td>
<td>2.64±0.81</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>12.90±0.01</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>5</td>
<td>MG</td>
<td>114.03±3.42</td>
<td>&lt;0.001</td>
<td>&lt;0.003</td>
<td>137.37±6.82</td>
<td>7.5±1.17</td>
<td>&lt;0.001</td>
<td>19.71±3.07</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>44.4±9.88</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>6</td>
<td>PU</td>
<td>70.52±12.43</td>
<td>&lt;0.001</td>
<td>&lt;0.003</td>
<td>87.56±8.82</td>
<td>7.5±1.17</td>
<td>&lt;0.001</td>
<td>19.71±3.07</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>44.4±9.88</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>7</td>
<td>CG</td>
<td>134.70±9.86</td>
<td>&lt;0.001</td>
<td>&lt;0.003</td>
<td>36.60±2.90</td>
<td>2.45±0.52</td>
<td>&lt;0.001</td>
<td>0.72±0.90</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>10.3±1.17</td>
<td>&lt;0.002</td>
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<tr>
<td>8</td>
<td>PB</td>
<td>10.35±2.99</td>
<td>&lt;0.001</td>
<td>&lt;0.003</td>
<td>64.07±3.03</td>
<td>0.01±0.01</td>
<td>&lt;0.001</td>
<td>0.22±0.03</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.01±0.01</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>9</td>
<td>OG</td>
<td>22.90±1.90</td>
<td>&lt;0.001</td>
<td>&lt;0.003</td>
<td>36.12±1.72</td>
<td>0.14±0.08</td>
<td>&lt;0.001</td>
<td>0.18±0.08</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.01±0.01</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>10</td>
<td>CL</td>
<td>27.95±5.77</td>
<td>&lt;0.001</td>
<td>&lt;0.003</td>
<td>43.70±2.88</td>
<td>0.15±0.07</td>
<td>&lt;0.001</td>
<td>0.64±0.11</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.01±0.01</td>
<td>&lt;0.002</td>
</tr>
</tbody>
</table>

Table 4: Amount of DOP isolated from each herbal drink mg/L

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>KA</th>
<th>HA</th>
<th>MD</th>
<th>AG</th>
<th>OG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of phthalate (DOP) isolated (mg/L)</td>
<td>12.53</td>
<td>30.72</td>
<td>7.84</td>
<td>36.22</td>
<td>15.76</td>
</tr>
</tbody>
</table>

Key: KA = Alomo HA: Herb Afrik MD: Mandingo AG: Agya Appiah OG: Ogidigidi

Table 5: Evaluation of Anti-microbial potency of Ghanaian herbal alcoholic bitters

<table>
<thead>
<tr>
<th></th>
<th>KA</th>
<th>HA</th>
<th>AG</th>
<th>MG</th>
<th>OP</th>
<th>PU</th>
<th>CG</th>
<th>PB</th>
<th>OG</th>
<th>CL</th>
<th>AMP</th>
<th>OXA</th>
<th>OTC</th>
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<tbody>
<tr>
<td>STAPHYLOCOCCUS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aureus. NCIMB12702</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ESCHERICHIA Coli.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>NCIMB12210</td>
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</tr>
</tbody>
</table>

Key: Negative: - Positive: +

Discussion

None of the drinks studied had a label displaying the major constituent of the drink, manufacture and expiry dates (Table 1).

A ten week study of the pH of Ghanaian herbal alcoholic bitters sampled revealed that these drinks are typically acidic. While two of the drinks started off as very acidic with pH between 1.0-2.5 (KA and PB) the drinks became less acidic with final pH 2.0-3.0 over the ten week study periods. The remainder of the drinks started off as slightly alkaline mixtures with typical pH of between 8.0-6.4 and after ten weeks, ended up as acidic mixtures with pH between 6.0-5.0. For simplicity, only the full results for KA is shown in Table 2 where the initial pH at all storage conditions is about 1.19 and all pH average out to about 2.46 at the end of the ten week study period.

The drinks KA, HA, MD, AG and OG, the five most patronized brands out of the ten (10) herbal alcoholic bitters sampled were investigated for any isolatable organics. However, it appears that, because each of these drinks are formulated from a combination of not less than three plant parts the organic composition of these drinks can be described as very complex. There are minute amounts of many phytochemicals present in the drinks but these molecules are not necessarily isolatable except for tannins and saponins (Figure 7 and 8). Since the manufacturers refuse to give information on the plants used for the formulation of each drink, it was difficult to do a detailed tracking of phytochemicals using the mass spectra acquired and hence
further study is required.

However, the drinks could partition in dichloromethane (DCM) to give a less polar fraction which on rotary evaporation gave a light yellow liquid. Repeated normal phase column chromatography led to the isolation of few milligrams of an oily compound which was characterized by mass spectrometry as di-n-octyl phthalate DOP (Table 4 and Figure 4 and 5). The distinction between DOP and bis(2-ethylhexyl) phthalate (DEHP) was achieved by inspection of a $^1$H NMR spectrum of DOP (not shown here). In order to confirm that the phthalates were indeed coming from the drinks and not from an experimental error (e.g. contaminated solvents), raw drink samples were subjected to High Resolution-Liquid Chromatography-Mass Spectrometry (HR-LC-MS) analysis without previously bringing them into contact with any laboratory solvents (Figure 7 and 8). Phthalates, predominantly DOP, were seen to elute in almost all the chromatograms at retention times of 20-21 minutes. The amount of DOP recovered from all the five drinks exceeded the WHO permissible limits of phthalates in bottled water which is 8µg/L. It turns out that the companies involved in the production of these drinks use water reservoirs like Sintex and Polytanks for their cold percolation processes (Figure 2 and 3). The drinks are most of the time kept for long periods in these water reservoirs until they are ready for bottling. Once ready for bottling, polyvinyl chloride (PVC) pipes transport the extracts to the bottling plant where the manufacture process continues. Hence, PVC pipes were purchased from Duraplast and Interplast the major companies involved in the production of these pipes. The material used for making Sintex tanks was also obtained from the company in Accra (Figure 2). These materials were found to contain phthalates amongst a mixture of other components (Figure 6). This result confirmed that a majority of phthalate contamination could come from the Sintex tanks and the PVC pipes amongst other manufacture parts through which the drinks are passed before the final processes of bottling and packaging.

The anti-microbial activity of all ten drink samples from the batches of drinks bought for the study was evaluated in triplicates against pathogenic bacteria strains; *Staphylococcus aureus* NCIMB12702 and *Escherichia coli* NCIMB12210. No inhibition zones were obtained for the raw drinks and various serial dilutions of all the herbal alcoholic drinks tested. The only inhibition zones measured after incubation were those for standard antibiotics (Table 5). The results showed that these herbal alcoholic drinks were inactive against the bacteria strains tested. The inability of the herbal alcoholic extracts to, on their own, inhibit bacterial growth suggests that if bacteria found their way into the bottle, they could flourish in the herbal alcoholic bitters which are normally not preserved in a refrigerator. Most bacteria grow best at neutral pH (6.5-7.0) however, acid loving bacteria known as acidophiles could tolerate pH as low as 1.0. Therefore, the low pH values measured could still support the growth of some bacteria. Microbes are ubiquitous in the environment thus microbial invasion of the drinks, once the seal of a bottle is broken is most likely to occur. Microbial invasion may lead to infections and microbial toxicity as a by-product of bacterial and fungal action on the rather heavy organic load of drinks.

Heavy metals were detected in almost all the ten drinks except in few cases where
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the metals were below detection limit (Table 3). The wide dispersion in data could be due to different herbal mixtures or plants each company employs in the product. On the other hand higher concentrations of trace metals observed in some drinks could also be the result of the soil or geology on which the herbs were collected besides other anthropogenic activities like mining, fertilizer application to soils etc. and air transport of chemicals and their deposition as wet/dry precipitation on plants.

Among the toxic elements (Hg, Cd, Se and Pb) examined, all the drinks showed no detectable levels except As, the only metalloid found in elevated concentrations in KA, OP, HA, AA, MG, PU and CG drinks. The presence of As can be attributed to the ability of As to adsorb to soil colloids and their subsequent absorption by plant roots [9, 10]. The principal causes of elevated soil As level might also be due to the historically widespread use of As compounds such as pesticides, mining, fossil fuel combustion and as part of phosphate fertilizers [9, 10]. The other drinks PB, OG and CL contained non-detectable levels of As. The metals Al, Mn, Fe, and Zn were contained in all the drinks while Cr and Cu were observed in only PU, CG, PB, OG and CL except OG where Cu level was below detection limit. In general, Al levels in all the drinks were very high exceeding the WHO recommended daily intake (RDI) of 0.2mg/L. It ranged from 10.35mg/L in PB to 134mg/L in CG. These high values could be due to the presence of Al in soils where plants were collected. In addition, Al occurs naturally in raw water, use of Al-based coagulants especially Al₂(SO₄)₃ always leads to an increase in treated water. Al from soils and this residual Al in treated water could also account for the high levels.

Additionally, Fe and Mn contents also exceeded WHO RDI of 15mg/L and 2mg/L respectively except in PU, PB, OG and CL drinks which had levels of Mn 0.88, 0.010, 0.14 and 0.15 mg/L respectively. These trace metal levels in the drinks could be the result of propensity for plants to accumulate and translocate them to edible and harvested parts depending on a large extent on soil and climatic factors [9]. However, in the case of Cu and Zn, the concentrations were below WHO RDI guideline values except for OP and MG where Zn concentration exceeded the permissible limits. Ni levels were below detection limit except OG which contained 0.26mg/L higher than expected 0.02mg/L RDI of WHO.

Conclusion

This study has shown that there are no specific chemical entities to which many of the desirable effects attributed to the intake of Ghanaian herbal alcoholic bitters can be assigned. Rather, the routine presence of phthalates can be considered as harmful since these compounds are known to cause carcinogenic, teratogenic and endocrine effects. Some phthalates have even shown reproductive and developmental toxicity in animal experiments and are known to affect semen production in men [11, 12]. In women, phthalates are known to increase the risk of breast cancer [13].

All the drinks analyzed were found to be acidic which is dangerous since our bodies constantly thrive to maintain our body pH at around 7.40 pH units. Maintaining proper body cell and tissue pH is critical for staying healthy and creating an inhospitable environment for cancer cells and viruses, bacteria and fungi to
multiply. Acidosis is the result of excess acidity and the implications are always detrimental to prolonged and healthy functioning of the body [14].

The dangers of heavy metal intake relates directly to the fact that, these substances cannot be metabolized and degraded. Hence, bioaccumulation is normally the end result leading to many disease conditions which are mostly characteristic to the particular metal under consideration. In general, heavy metal poisoning can cause our mental functions, energy, nervous systems, kidneys, lungs and other vital organ functions to decline [15].

Despite the perceived benefits, it is evident that patrons of these alcoholic bitters may be ingesting contaminants which may have some health implications.

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