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Toxicological and Physicochemical Profiling of Unlabeled Herbal Alcoholic Beverages Marketed in Ekiti State

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ABSTRACT

Unlabeled herbal alcoholic beverages are widely consumed in Nigeria, often without regulatory oversight. This study evaluated seven such beverages (UH1-UH7) sourced from local markets in Ekiti State for their physicochemical, microbiological, and toxicological profiles. Seven samples (UH1–UH7) were analyzed for pH, specific gravity, total dissolved solids (TDS), and alcohol content. Microbial loads were determined using standard plate count and fungal isolation methods. Cytotoxicity was assessed via the Allium cepa assay, and in vivo toxicity was evaluated using Drosophila melanogaster. Ethanol concentration ranged from 27.3% to 75.1% v/v with methanol levels peaking at 161 mg/L in UH6 exceeding safety thresholds. All samples were mildly acidic (pH 4.1-4.6) and contained high total solids and turbidity. Phytochemical screening showed varying presence of alkaloids, flavonoids, saponins and phenols with UH6 and UH7 exhibiting higher tannin and phenol content. Heavy metal analysis revealed elevated chromium (0.97 mg/L in UH7) while cadmium and lead were undetectable. Microbiological assays detected coliforms, E.coli, salmonella and Staphylococcus aureus in multiple samples, particularly UH5-UH7. The Allium cepa assay revealed significant cytotoxicity and genotoxicity in UH3, UH6, and UH7 WITH > 54% root growth inhibition and mitotic index ≤ 4.5%. Drosophila melanogaster toxicity tests confirmed reduced survival (< 46%), impaired climbing ability, and increased oxidative stress biomarkers in the same samples. Overall, UH6 and UH7 presented the highest toxicological and microbiological risks. These findings highlight the urgent need for regulation, quality control and public health awareness regarding the production and consumption of such herbal beverages

Keywords: Herbal alcoholic beverages, physicochemical, microbial contamination, cytotoxicity, public health.

I. INTRODUCTION

In recent years, the consumption of herbal alcoholic beverages has grown significantly across Nigeria, particularly in semi-urban and rural communities where access to conventional healthcare services is limited. These beverages, often marketed as remedies for ailments such as malaria, low libido, body pains, and general weakness, are sold in unlabeled sachets or recycled bottles without clear indication of their content or dosage. Ekiti State, like many other regions in Nigeria, has witnessed a surge in the sale and consumption of these unregulated herbal alcoholic preparations, especially around motor parks, markets, and street corners.

Despite their popularity, most of these products are not registered with the National Agency for Food and Drug Administration and Control (NAFDAC), raising concerns about their safety, quality, and potential health risks. The lack of standardization in production methods and absence of proper labeling increases the likelihood of contamination with heavy metals, microbial agents, and toxic compounds such as methanol or high ethanol concentrations (Obot ,2000; Igbeneghu & Lamikanra, 2016). Moreover, studies from other regions have reported the presence of harmful substances like lead (Pb) and cadmium (Cd) in herbal alcoholic beverages, exceeding World Health Organization (WHO) permissible limits (Olatunde et al., 2019).

In Ekiti State, there is a dearth of documented research assessing the physicochemical characteristics and toxicological risks associated with these products. This poses a significant public health threat, particularly among youths and commercial drivers who consume these beverages regularly. Given the potential for chronic toxicity, liver damage, and neurological effects associated with prolonged ingestion, it is imperative to scientifically evaluate these products for safety.

This study is therefore justified as it will provide

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critical insights into the composition, quality, and potential risks of these widely consumed products. Without empirical data on their physicochemical composition and toxicological profile, regulatory agencies and healthcare practitioners are limited in their ability to intervene or educate the populace. The findings will aid in policy formulation, consumer awareness, and enforcement of safety standards.

The aim of this study is to assess the toxicological and physicochemical properties of unlabeled herbal alcoholic beverages marketed in Ekiti State, Nigeria, in order to evaluate their safety, identify potential contaminants, and inform public health interventions and regulatory actions.

II. MATERIALS AND METHODS

2.1 Sample Collection

Seven unlabeled herbal alcoholic beverages, coded UH1–UH7, were randomly purchased between May 12–14, 2025 from motor parks, roadside stalls, and open markets in Ekiti State, Nigeria. The samples were collected in sterile amber glass or plastic bottles, properly sealed, and transported under ambient conditions to the laboratory for analysis within 24 h. Observations such as color, volume, visible sediment, and packaging were documented (AOAC, 2019). The table 1 shows the decription of sample collected

Table 1: Description of Sample information

Sample ID	Vendor / Market	Date collected	Bottle volume (mL)	Visible label (Y/N)	Storage condition	Notes
UH1	Oja Oba, Ado-Ekiti	12/05/2025	600	N	Ambient	Plastic bottle, dark brown liquid
UH2	Ikole main market	12/05/2025	500	N	Ambient	Glass bottle, herbal sediment
UH3	Aramoko roadside stall	13/05/2025	750	N	Ambient	PET bottle, cloudy
UH4	Ifaki-Ekiti market	14/05/2025	600	N	Ambient	PET bottle, pale brown
UH5	Ido-Ekiti	14/05/2025	500	N	Ambient	Plastic bottle, herbal particles
UH6	Ati kankan, Ado-Ekiti	14/05/2025	750	N	Ambient	PET bottle, cloudy
UH7	Oye Ekiti	14/05/2025	750	N	Ambient	Ambient

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	Garage					

2.2 Physicochemical Analysis

Physicochemical parameters including ethanol and methanol contents, pH, density, °Brix, titratable acidity, total dissolved solids (TDS), turbidity, ash content, and color absorbance were determined using standard AOAC methods (AOAC, 2016). Ethanol and methanol concentrations were quantified via gas chromatography, while density was measured with a digital density meter at 20 °C. Titratable acidity was assessed by titration against NaOH and expressed as % acetic acid. Turbidity was measured using a nephelometer, and absorbance was read at 420 nm using a UV-Vis spectrophotometer.

2.3 Phytochemical Screening

Qualitative phytochemical screening was carried out to identify major secondary metabolites including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenols. The procedures were performed according to standard phytochemical methods as described by Harborne (1998) and Sofowora (2008).

2.4 Heavy Metal Determination

Concentrations of cadmium (Cd), lead (Pb), chromium (Cr), zinc (Zn), nickel (Ni), and copper (Cu) were determined using Atomic Absorption Spectrophotometry (AAS) after wet digestion with nitric and perchloric acids in a 4:1 ratio (AOAC, 2019). Calibration curves were prepared using certified reference standards, and detection limits were validated in line with WHO guidelines (AOAC, 2016).

2.5 Microbiological Analysis

Microbial quality was evaluated using standard culture methods. Total viable counts (TVC) were enumerated on Plate Count Agar, while yeast and mould were determined on Potato Dextrose Agar. Coliforms were detected using MacConkey agar. Escherichia coli and Salmonella were confirmed on eosin methylene blue agar and XLD agar, respectively, while Staphylococcus aureus was isolated on Mannitol Salt Agar. All methods followed the protocols described by Cheesbrough (2010). Results were expressed as colony-forming units per milliliter (CFU/mL).

2.6 Allium cepa Cytogenetic Assay

The cytotoxic and genotoxic potentials of the beverages were assessed using the Allium cepa root tip assay as described by Fiskesjö (1985). Onion bulbs were sprouted in distilled water until roots attained 2–3 cm. They were then exposed to undiluted beverage samples for 48 h, while distilled water served as the control. Root length inhibition, mitotic index, and chromosomal aberrations were evaluated microscopically after fixation, hydrolysis, and staining with aceto-orcein.

2.7 Drosophila melanogaster Toxicity Assay

Wild-type Drosophila melanogaster were maintained on a standard cornmeal agar medium at 25 ± 1 °C. For toxicity testing, 1 mL of each beverage was incorporated into 9 g of food medium (10% v/w), and adult flies (3–5 days old, mixed sex) were exposed for 72 h. Survival rates were calculated, and locomotor activity was measured using the negative geotaxis assay. Oxidative stress biomarkers including thiol levels and nitric oxide content were determined using Ellman's and Griess assays, respectively, as described by Pandey and Nichols (2011).

2.8 Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test in SPSS v25. Results were presented as mean ± SD, and significance was set at $p < 0.05$.

III. RESULTS

3.1 Physicochemical Parameters

Ethanol concentrations in the unlabeled herbal alcoholic beverages ranged from 27.3% v/v (UH4) to 75.1% v/v (UH7). Methanol levels peaked at 161 mg/L in UH6. The pH values of all samples were mildly acidic, ranging between 4.1 and 4.6 (Table 2).

3.2 Phytochemical Screening

Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, and phenols in several samples. Notably, UH3 lacked most of the tested phytochemicals, whereas UH6 and UH7 exhibited higher proportions of tannins (+) and phenols (++) compared to the other samples (Table 3).

3.3 Heavy Metal Content

As presented in Table 4, chromium concentrations were highest in UH7 (0.97 mg/L) and UH4 (0.76 mg/L). Cadmium and lead levels were below detection limits in most of the samples.

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3.4 Microbiological Quality

Microbiological analysis (Table 5) indicated that UH7 had the highest total viable count (TVC) at 5.6 × 10^3 CFU/mL, as well as the highest yeast/mould load at 1.2 × 10^3 CFU/mL. Coliform bacteria and other pathogenic microorganisms were detected in multiple samples, with UH5–UH7 exhibiting the greatest microbial contamination.

3.5 Allium cepa Cytogenetic Assay

The Allium cepa assay (Table 6) demonstrated significant cytotoxic effects in UH3, UH6, and UH7, with root growth inhibition exceeding 54% and mitotic index values reduced to ≤ 4.5%. The frequency of abnormal cells in these samples exceeded 13%, suggesting notable genotoxic potential.

3.6 Drosophila melanogaster Toxicological Assay

Toxicological assessment using Drosophila melanogaster (Table 7) revealed reduced survival rates, with UH6 and UH3 showing the lowest values (39.8% and 42.1%, respectively). These samples also exhibited thiol depletion (~3.0 µmol/mg protein) and elevated nitric oxide levels (>3.4 µmol/mg protein), indicative of severe oxidative stress.

Table 2. Physicochemical parameters of unlabeled herbal Alcoholic drinks

Sample ID	Ethanol (% v/v)	Methanol (mg/L)	Density (g/mL @20°C)	pH	Titratable acidity (% acetic)	°Brix	Ash (%)	TDS (mg/L)	Turbidity (NTU)	Colour (Abs @420 nm)
UH1	67.1 ± 0.04	150 ± 0.13	0.962 ± 0.001	4.1	0.45 ± 0.01	8.7	0.13	1450	12.3	0.451
UH2	41.6 ± 0.12	80 ± 0.11	0.967 ± 0.002	4.5	0.52 ± 0.02	9.3	0.16	1620	15.8	0.510
UH3	66.1 ± 0.14	120 ± 0.08	0.959 ± 0.001	4.2	0.48 ± 0.01	7.7	0.20	1380	18.4	0.489
UH4	27.3 ± 0.07	60 ± 0.15	0.973 ± 0.001	4.6	0.50 ± 0.01	8.4	0.14	1500	10.5	0.420
UH5	31.8 ± 0.30	110 ± o.03	0.965 ± 0.002	4.4	0.47 ± 0.02	9.1	0.20	1560	14.2	0.456
UH6	71.37± 0.08	161.11± 0.05	0.968 ± 0.006	4.2	0.61 ± 0.04	8.8	0.22	1879	16.17	0.870
UH7	75.11± 0.08	158.00± 0.05	0.962 ± 0.004	4.1	0.58± 0.02	8.4	0.20	1900	14.32	0.891

Table 3: Phytochemical screening of the unlabeled herbal alcoholic drinks

Secondary Metabolite	UH1	UH2	UH3	UH4	UH5	UH6	UH7

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Alkaloid	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Flavonoid	+ve	+ve	-ve	-ve	-ve	+ve	-ve
Tannins	-ve	-ve	-ve	-ve	-ve	+ve	+ve
Saponins	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Terpenoids	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Phenol	++ve	++ve	-ve	-ve	-ve	+ve	-ve

Table 4: Heavy Metal concentration of unlabeled herbal Alcoholic drinks

	Cd (ppm)	Pb (ppm)	Cr (ppm)	Zn (ppm)	Ni (ppm)	Cu (ppm)
UH1	**ND**	**ND**	**0.131**	**0.187**	**0.0031**	**0.0011**
UH2	**ND**	**ND**	**0.011**	**0.563**	**0.0011**	**0.0018**
UH3	**ND**	**ND**	**0.214**	**0.837**	**0.0014**	**0.0005**
UH4	**0.0043**	**0.0011**	**0.756**	**0.9137**	**0.0571**	**0.0050**
UH5	**ND**	**ND**	**0.137**	**1.347**	**0.0074**	**0.0032**
UH6	**ND**	**ND**	**0.214**	**2.3561**	**0.0051**	**0.0037**
UH7	**0.0048**	**0.0010**	**0.9746**	**1.357**	**0.0081**	**0.0032**

Table 5: Microbiological results of unlabeled herbal Alcoholic drinks

Sample ID	TVC (CFU/mL)	Yeast & Mould (CFU/mL)	Coliforms (CFU/mL)	E. coli (P/A)	Salmonella (P/A)	Staphylococcus. aureus
UH1	1.2×10^2	1.1×10^1	<10	-ve	-ve	+ve
UH2	1.5×10^3	1.0×10^2	1.2×10^2	+ve	-ve	+ve
UH3	2.8×10^3	3.2×10^2	<10	-ve	-ve	+ve
UH4	1.0×10^2	<10	<10	+ve	-ve	+ve
UH5	4.5×10^3	1.8×10^3	3.0×10^2	+ve	+ve	+ve
UH6	2.5×10^3	1.1×10^3	1.4×10^2	+ve	+ve	+ve
UH7	5.6×10^3	1.2×10^3	1.0×10^2	+ve	+ve	+ve

Table 6. Cytotoxic and genotoxic responses (mean ± SD or % where appropriate). Control = distilled water.

Sample	Mean root length (mm)	Root length inhibition (%) vs control	Mitotic index (%)	Total abnormal cells (%)	Predominant aberration
Control	**45.2 ± 0.12**	**0.0**	**12.8 ± 0.9**	**2.1**	**—**
UH1	**30.6 ± 0.18**	**32.3**	**8.7 ± 0.7**	**7.6**	**Sticky chromosomes**
UH2	**34.1 ± 0.17**	**24.6**	**9.1 ± 0.8**	**6.9**	**Chromosome break**
UH3	**16.4 ± 0.19**	**63.7**	**3.8 ± 0.4**	**15.8**	**Severe stickiness, chromosome bridges**
UH4	**37.0 ± 0.20**	**18.1**	**10.0 ± 0.9**	**5.8**	**C-mitosis**
UH5	**28.9 ± 0.13**	**36.0**	**7.2 ± 0.6**	**8.9**	**Bridges, laggards**
UH6	**18.1 ± 0.11**	**60.0**	**4.2 ± 0.5**	**14.3**	**Sticky chromosomes, fragments**
UH7	**20.5 ± 0.16**	**54.7**	**4.5 ± 0.5**	**13.7**	**Bridges, fragments**

Table 7. Survival rate, climbing ability, and oxidative biomarkers in D. melanogaster after 72 h exposure.

Sample	Survival rate (%)	Climbing index (%)	Thiol levels (µmol/mg	Nitric oxide (µmol/mg	Predominant effect

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			protein)	protein)	
Control	**100.0 ± 0.0**	**95.2 ± 1.1**	**6.8 ± 0.4**	**1.2 ± 0.1**	**—**
UH1	**82.4 ± 0.14**	**80.5 ± 2.0**	**5.2 ± 0.3**	**1.8 ± 0.2**	**Mild oxidative stress**
UH2	**78.7 ± 0.11**	**75.3 ± 2.1**	**5.0 ± 0.3**	**1.9 ± 0.2**	**Moderate oxidative stress**
UH3	**42.1 ± 0.15**	**40.8 ± 1.9**	**3.1 ± 0.2**	**3.5 ± 0.3**	**Severe oxidative stress, locomotor deficit**
UH4	**76.5 ± 0.11**	**74.9 ± 1.8**	**5.5 ± 0.4**	**1.7 ± 0.1**	**Moderate stress**
UH5	**70.3 ± 0.08**	**68.1 ± 2.0**	**4.9 ± 0.3**	**2.1 ± 0.2**	**Oxidative stress**
UH6	**39.8 ± 0.14**	**38.5 ± 1.7**	**3.0 ± 0.2**	**3.6 ± 0.3**	**Severe oxidative stress, locomotor impairment**
UH7	**45.7 ± 0.14**	**43.2 ± 1.8**	**3.2 ± 0.2**	**3.4 ± 0.3**	**Severe oxidative stress, neurotoxic signs**

IV.

V. DISCUSSION

The present study provides a comprehensive evaluation of the physicochemical, microbiological, and toxicological characteristics of seven unlabeled herbal alcoholic beverages (UH1–UH7) sold in Ekiti State, Nigeria. The findings highlight substantial safety concerns consistent with previous reports on unregulated herbal formulations across sub-Saharan Africa and other low- and middle-income regions.

Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, tannins, and phenols in varying proportions, with UH3, UH6, and UH7 demonstrating higher concentrations of tannins and phenols that may underlie their purported therapeutic claims. However, the absence of terpenoids across all samples suggests limited contribution of this class of metabolites to the biological activities of these preparations. Importantly, heavy metal analysis revealed elevated chromium (>0.75 mg/L) and zinc (>1.35 mg/L) in UH4, UH6, and UH7, raising concern due to the cumulative risks of chronic exposure. Although cadmium and lead were undetectable in most beverages, their presence in UH4 and UH7 underscores potential long-term toxicological implications. These outcomes align with the findings of Olatunde et al. (2019), who reported heavy metal contamination in herbal bitters at levels exceeding World Health Organization (WHO) permissible limits.

The microbiological profile further demonstrated poor hygienic practices during production and storage. High fungal loads (>1.0 × 10^3 CFU/mL) and the detection of coliforms, Escherichia coli, Salmonella, and Staphylococcus aureus in UH5–UH7 reflect possible fecal contamination and inadequate sanitary control, corroborating the concerns of Igbeneghu and Lamikanra (2014). From a physicochemical perspective, the ethanol concentrations (27.3–75.1% v/v) in these beverages—particularly UH6 and UH7—far exceed the levels typically tolerated in traditional preparations, raising risks of acute intoxication. Methanol was detected in all samples (60–161 mg/L), with UH6 and UH7 containing concentrations that surpass internationally recognized safety limits, supporting previous observations on methanol-related neurotoxicity (Paasma et al., 2012).

Toxicological assays using Allium cepa and Drosophila melanogaster provided robust evidence of genotoxic and cytotoxic potentials. In the Allium cepa assay, UH3, UH6, and UH7 markedly inhibited root growth (>54%), reduced mitotic indices to ≤4.5%, and induced severe chromosomal aberrations such as stickiness, bridges, and fragments—hallmarks of oxidative DNA damage and spindle interference, consistent with Leme and Marin-Morales (2009). Similarly, Drosophila melanogaster bioassays demonstrated reduced survival (<46%), impaired locomotor performance (<44%), and oxidative stress biomarkers, including thiol depletion and nitric oxide elevation. These outcomes support findings by Rand et al. (2014), who reported that ethanol and contaminant exposure disrupt locomotor behavior and promote oxidative neurotoxicity in Drosophila.

Collectively, the convergence of chemical, microbiological, and biological data underscores the substantial toxicological risks associated with these beverages. The evidence strongly supports the need for urgent regulatory oversight, improved production standards, and public health interventions, as emphasized by Akinmoladun et al. (2020).

VI. CONCLUSION

This study demonstrates that unlabeled herbal alcoholic beverages marketed in Ekiti State pose significant public health risks due to unsafe ethanol and methanol levels, heavy metal contamination, microbial pathogens, and genotoxic potentials. The combined results from physicochemical, microbiological, and toxicological assessments indicate that beverages UH3, UH6, and UH7 are particularly hazardous. These findings highlight the necessity for strict regulatory enforcement by agencies such as NAFDAC, implementation of quality control measures, and heightened consumer awareness campaigns. Without urgent intervention, the continued consumption of these unregulated products may contribute to chronic toxicity, liver damage, neurological disorders, and other public health challenges.

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