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Metabolic Profiles and Physiochemical Evaluation of Selected Energy Drinks in Common Use in Abakaliki, Ebonyi State Using Potatoes Membrane as an Absorbent

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Abstract

Energy drink consumption has shown to be positively associated with high-risk behaviour. In this study, the physiochemical and metabolic profiling of four energy drinks were evaluated and the parameters assessed include: pH which showed slightly acidic and ranged from 4.47-5.18; turbidity ranged from 85.35 - 126.22 NTU; total dissolved solids (TDS) ranged from 380.22-956.21 mg/L; total suspended solid (TSS) concentrations were found to be 320.25 mg/L and conductivity ranged from 1035.01-1240.39 µs/cm. All of the above values were within the permissible limit of Food and Drug Administration (FDA). However, iron level of energy drinks ranged from 1.502 x 10^{-2} mg/L-1.930 x 10^{-2} mg/L, while the calcium concentration ranged between 1.24 x 10^{-1}- 1.73 x 10^{-1} mg/L. Potassium concentrations ranged from 12. 40 x 10^{2}-17.00 x 10^{2} mg/L which were a little lower than the recommended daily intake (RDI). Sample A and D revealed magnesium values of 1.9 x 10^{-1} mg/L and 1.8 x 10^{-1} mg/L with control at 8.1 x 10^{-1}, while the chloride content ranged from 4.91 x10^{-2} mg/L-5.75 x 10^{-2} mg/L control at 4.487 x 10^{2}. All the samples analyzed have the obtained values within the permissible limit, set by the regulatory agencies. The spectral analysis by GS-MS revealed the presence as well as the concentration of the following compounds: 4-methyl-2-propyl-furan (2.547%), 1-(hexyloxy)-3-methyl-Hexane (0.09%), Glutaric acid, 2-ethylhexyl 4-chloro-2-methoxyphenyl ester (0.082%), 2,3-dihydro-3,5-dihydroxy-6-methyl- 4H-Pyran-4-one (4.945%), Benzoic acid (0.876%), 3,5-dihydroxy-2-methyl- 4H-Pyran-4-one (0.0210%), 5-Acetoxymethyl-2-furaldehyde (0.156%), 5-Hydroxymethylfurfural (71.627%), Octanoic acid (1.152%), 2,6-Heptadienal, 2,4-dimethyl-(0.288%), Oxacyclooctadec-3-en-2-one, 5,6,7-trihydroxy-18-methyl- (2.711%), Caffeine (3.075%), 5-Methyl-N-(3-nitrophenyl)-2-furamide (0.098%). The percentage ion concentration of caffeine in all the samples were a little higher than the FDA set standard of 400 mg per day but majority of the compounds identified were metabolic products of sugar degradation. However, regulatory authorities saddled with monitoring the quality of food and drugs should periodically ascertain these oversight functions, for maintenance of food quality.

Keywords: Caffeine content, Energy drink, Evaluation, Metabolic profiles, Physiochemical.

Introduction

Energy drinks are reported to contain high amounts of caffeine and other legal stimulants [1]. The market for energy drinks expanded by 80% in 2016, with young adults making up the majority of consumers [2]. This is because the beverages can increase vitality, physical stamina, improve concentration and reaction speed [3]. According to recent survey Children and young adults use energy drinks for various reasons, inducing but not limited to sleep deprivation, fun at parties [4], and to elevate one's social standing or lessen the depressing effects of alcohol [5,6]

Health professionals, however, are concerned that these drinks and the drinking habits of the intended audience may actually have negative health effects [7]. In a recent study, heavy energy drink consumption was linked to hospitalization of people with pre-existing mental illness [8] and new onset seizures in four patients [9]. Concerns have been raised about the general health and wellbeing of these consumers due to the popularity of energy drinks and the rise in their use among young adults [10]. Studies on caffeine's effects on the cardiovascular system indicate that it probably affects hemodynamic parameters [11,12] and recent work by [13] revealed that healthy adults who consume up to 400 mg of caffeine per day do not experience any negative side effects. However, energy drinks with a higher caffeine content may be avoided by at-risk groups, such as children and women of reproductive age [11]. The caffeinated energy drinks had caffeine concentrations ranging from 141.1 mg/serving to zero [14]

Al-Mayaly [15] reported the presence of heavy metals in 20 samples of various artificial fruit juices sold in Iraqi, while [16] examined the pH, conductivity, turbidity, total dissolved solids, level of bitterness, reducing sugar, vitamins A and C, and minerals of five well-known commercial brands of malt beverages made in Nigeria. These beverages had an acidic pH between 4.4 and 4.6, TDS at 1480 mg/L, conductivity at 2.93 µS/cm and mineral content such as calcium, sodium, iron, zinc, manganese, and nickel were all very low. Monitoring pH levels in beverages is important because unbalanced drinks can lead to tooth decay, an exposure to metallic chemicals and a weakening of the immune system [17]. Energy drinks contains ions because of dissolved mineral matter in it and do have high electrical conductivity [18]. Maduabuchi *et al*. [19] reported the presence of iron in canned and non-canned beverages at 0.020–2.460 mg/L and 0.020–2.090 mg/L respectively, while manganese levels were observed at 0.001-0.730 mg/L, but non-canned beverages showed values of 0.001-0.209 mg/L. However, nickel concentrations in canned and non-canned beverages ranged from 0.013 to 0.993 mg/L and 0.009 to 0.938 mg/L, respectively in 50 Nigerian beverages. Bengol *et al.* [20] used ICP-OES) method to qualitatively assess metal content of beverages. The result depicts the mean concentrations of copper at 0.037 mg/kg, zinc at 0.070 mg/kg, iron at 0.143 mg/kg, and cadmium in soft drinks at 0.005 mg/kg respectively. In this study we attempt to evaluate the physicochemical prosperities and metabolic profiles of a selected energy drinks that are frequently consumed in Abakaliki, Ebonyi State, Nigeria.

Materials and Methods

Sample collection

Four (4) brands of energy drink liquid samples were randomly purchased from the market in Abakaliki, Ebonyi State, Nigeria in May, 2022 and analyzed.

The samples were refrigerated prior to analysis. The samples were labeled A, B, C, D respectively.

GC-MS analysis of the sample

Using a Perkin-Elmer gas chromatograph, clarus 500 system, and GC interfaced to a mass spectrometer equipped with an Elite-1 fused silic capillary column (30 mm X 25 mm1D X 1 μMdf, made of 100% dimethyl polysiloxane). An electron ionization system with an ionizing energy of 70 electron volts was used for the gas chromatography-mass spectrometric detection. A gas chromatograph was used to separate the sample's constituent parts according to their physical and chemical characteristics after the drinks were added. Following the separation of the components, the mass spectrometer ionized the compounds and measured the mass-to-charge ratio. The resulting mass spectra were compared to a database of known compounds in order to identify the sample's constituent parts. The identified compounds' name, molecular weight, structure, percentage ion concentration (%tic), and activity were determined.

Analysis of Physiochemical Parameters

Determination of pH, conductivity, turbidity and total dissolved solid (TDS)

A JENWAY 3505, UK digital pH meter was used to measure the pH of each of the energy drink sample, placed in a 100-milliliter volumetric flask. A digital turbidity meter (HACH DR/890, USA) probe was inserted and its turbidity values were recorded, the conductivity meter probe was inserted and its conductivity values were recorded, and the TDS/conductivity meter probe was inserted and its TDS, TSS values were recorded, in that order.

X-ray fluorescence of the drink samples

The XRF method depends on fundamental principles that are common to several other instrumental methods involving interactions between electron beams and x-rays with samples

This was achieved by using modified potatoes membrane as an absorbent. The potatoes tubers were peeled and cavity of 3-4 cm in diameter created within the tuber, it was then soaked in 0.01M NaOH. Thereafter it was removed and allowed to dry at an ambient temperature of (25 ^oC) for 6 hours. The liquid samples were then introduced into the moist potatoes cavity and allowed to percolate through a potato's membrane. The potatoes severed as an absorption membrane, allowing the filtrate to pass through, while trapping the dissolved mineral elements. The process was repeated thrice, thereafter solid sample obtained were dried at room temperature, grinded into powder and irradiated with high energy X-rays, Mastersizer 3000, (Malvern Pananalytical UK).

Results and Discussion

Physicochemical Parameters of Samples

Table 1: pH, Temperature, Conductivity, Turbidity, Total Dissolved Solids (TDS), and Total Suspended Solids (TSS) of the sampled energy drinks

S/No	Parameters	Sample A	Sample B	Sample C	Sample D
1	pH	4.63	5.18	4.47	4.54
2	Temp (°C)	34.30	31.30	33.30	31.15
3	Turbidity (NTU)	126.22	94.33	85.35	98.23
4	Conductivity($\mu s \cdot cm^{-3}$)	1035.01	1240.39	1160.02	1160.14
5	TDS (mg/L)	460.54	956.21	660.22	380.22
6	TSS (mg/L)	320.25	210.42	260.10	360.31

The pH of the samples ranged from 4.47 -5.18 as depicted in Table 1. Sample C has the lowest value (4.47), while sample B has the highest pH value of 5.18. The average pH values recorded for all the samples are within FDA limit. However, these results were higher than pH values of 2.75 – 3.66 reported elsewhere by [21] for soft and energy drinks. They were within the pH ranged of 4.2 – 6.3 reported by [22] for local beverages in Nigeria, and had similarities with pH values of 4.2 – 6.3 reported by [16] for malt beverages. The pH of all the samples is acidic. The reason behind the low pH values of these beverages may be attributed to the CO_2 gas or the presence of other organic acids used as preservatives [17]. Drinking acidic beverages over a long period can erode tooth enamel and predispose the consumer to dental disease [23, 17].

The pH of the analyzed energy drinks is in the range recommended by FDA for caffeinated drinks and coffee of 4.7 and 6.0 [24].

The turbidity of the analyzed samples ranged from 85.35 – 126.22 NTU with sample C having the least and sample A having the highest turbidity. These are lower than results reported by [24] for malt drinks which had turbidity values above detection limits (>1000NTU).

While the conductivity of the sampled energy drinks ranged from 1035.01-1240.39 µs/cm. These values were similar to values reported by [16] for nutritional content of popular malt drinks. Sample A is less conductive while sample B has the highest conductivity. Conductivity is a useful indicator of the salinity or total salt content of foods. These results are not surprising since energy drinks contain high level of dissolved salts. The conductivity values for all the samples are higher than the WHO guideline values of 1000 $\mu s \cdot cm^{-3}$.

The total dissolved solids (TDS) ranged from 380.22 – 956.21 mg/L whereas the total suspended solid (TSS) concentrations were found to be 320.25, 210.42, 260.10 and 360.31 mg/L for samples A to

D respectively. These values for TDS were within the range of 327.37 – 1480 mg/L reported by [16] for malt drinks. Sample B had the highest TDS while sample D had the least TDS. Beverages with high values of TDS are likely to contain metals (essential and toxic) at high concentrations which may cause adverse health effects when consumed. These values obtained for TDS in all the samples were found below the WHO standard of 2000 mg/L for surface water [25].

The essential elements concentration in the selected energy drinks obtained from x-ray fluorescence analysis using potatoes membrane as an absorbent are as depicted in Table 2.

Table 2: Iron, Calcium, Potassium, Magnesium, Phosphorus and Chloride concentrations of the energy drink samples

Samples	Iron (mg/L)	Calcium (mg/L)	Potassium (mg/L)	Magnesium (mg/L)	Phosphorus (mg/L)	Chloride (mg/L)
A	1.930×10^{-2}	1.275×10^{-1}	14.77×10^{2}	1.9×10^{-1}	1.58×10^{-2}	5.390×10^{-2}
B	1.502×10^{-2}	1.444×10^{-1}	13.23×10^{2}	3.8×10^{-1}	1.896×10^{-2}	5.754×10^{-2}
C	1.575×10^{-2}	1.73×10^{-1}	12.40×10^{2}	1.1×10^{-1}	3.636×10^{-2}	5.52×10^{-2}
D	1.599×10^{-2}	1.24×10^{-1}	17.00×10^{2}	1.8×10^{-1}	1.998×10^{-2}	4.91×10^{-2}
Control	1.603×10^{-1}	1.492×10^{-1}	23.90×10^{2}	8.1×10^{-1}	1.987×10^{-1}	4.487×10^{2}

Iron is important in many biological processes, although excessive iron intake has been implicated in colorectal cancer [26]. In this study iron level of the sampled energy drinks ranged from 1.502×10^{-2} mg/L – 1.930×10^{-2} mg/L, while the control gave 1.603×10^{-1} mg/L. These values were within the limits as compared to 0.020 – 2.090 mg/L for non canned and 0.020 – 2.460 mg/L for canned beverages reported by [19]. All the sampled energy drinks had iron concentration lower than 0.03 mg/L recommended limit [27]

Calcium concentration of the analyzed energy drinks ranged between 1.24×10^{-1} - 1.73×10^{-1} mg/L, with sample D having the least and sample C the highest value, while control had values of 1.492×10^{-1} mg/L. The calcium concentration of energy drinks was low compared to 0.28 – 262 mg/L reported by [23] malta drink. Calcium is necessary for blood clotting, stabilizes many body functions and is thought to assist in preventing bowel cancer [28] It has a natural calming and tranquilizing effect and is necessary for maintaining a regular heartbeat and the transmission of nerve impulses. The required daily intake is 1,000 mg/day and 1,200 mg per day depending on age [29].

Potassium concentrations of the energy drinks were relatively low compared to the recommended daily intake (RDI) of potassium [27]. It ranged from 12.40×10^{2} mg/L -17.00×10^{2} mg/L as shown in Table 2. The RDI of potassium ranged between

1600-5000 mg/day. Only sample D had a concentration of 17.00 x 10^2 mg/L which is within the recommended RDI of potassium. Potassium is the major intracellular ion, intimately related to sodium movement out of the cell via Na/K ATPase. As such, it participates in maintaining normal membrane potential in cells. Potassium is also a major factor in osmotic fluid dynamics in the body. Renal control mechanisms keep blood levels within a narrow range.

Samples A and D had magnesium contents of 1.9 x 10^{-1} mg/L and 1.8 x 10^{-1} mg/L, respectively, while samples C and B had magnesium contents ranging from 1.1 x 10^{-1} mg/L to 3.8 x 10^{-1} mg/L. The control had the highest value at 8.1 x 10^{-1} mg/L. However, Abdelazim [30] reported that the magnesium content of carbonated drinks as 3.98±0.01 and 64.90±0.01 mg/100mL. The phosphorus content of the sampled energy drinks ranges from 1.998 x 10^{-2} – 3.636 x 10^{-2} mg/L with the control having the lowest value 1.987 x 10^{-1} mg/L. However, the energy drinks under investigation have a chloride content between 4.91 and 5.75 x 10^2 mg/L. At 5.75 x 10^2 mg/L, sample B had the highest value and sample D the lowest. At 4.48 x 10^2 mg/L, the control gave the lowest value. The presence of calcium chloride may be implicated. In the human body, calcium chloride serves as an electrolyte and most sports drinks contain $CaCl_2$ which gives them their distinctively salty taste [31]

Spectral data of organic compounds identified in samples A: The compounds identified through mass spectrometry attached to a gas chromatograph were shown on the chromatograph Fig. 1 and the results are tabulated for the various samples in Table 3. The organics with their retention time (RT), percentage ion concentration (% tic), molecular formula and molecular weight (MW) in the samples are presented in Tables 3. A total of Twenty-five (25) compounds were tentatively identified.

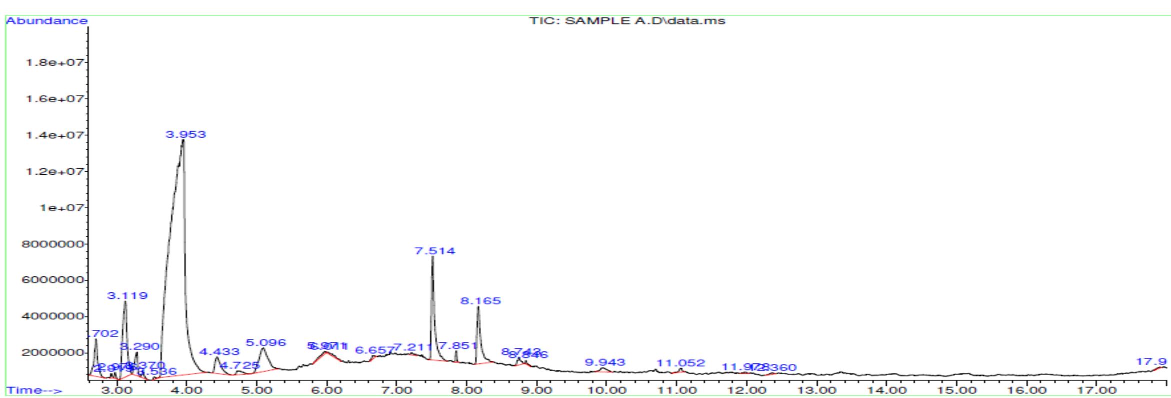
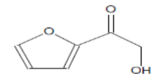
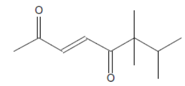
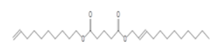
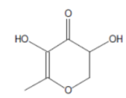
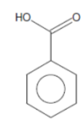
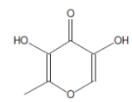
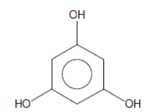
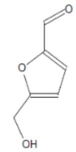
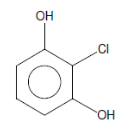
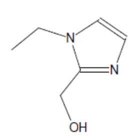
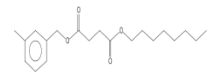
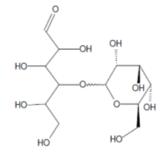
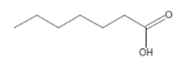
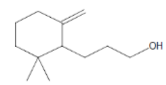
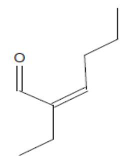
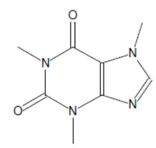
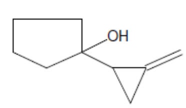
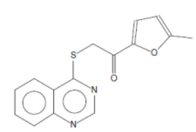
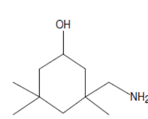
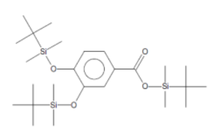
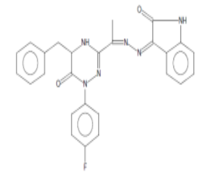
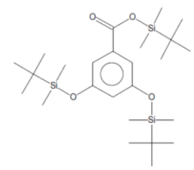


Fig. 3.1. GC-MS profile of organic compounds identified in sample A.

Table 3: Organic components detected in sample A

S/N	Name of Compound	Mol. Formular	Mol. Wt (g)	RT (min)	% TIC	Structure	Activity
1	Furyl hydroxymethyl ketone	$C_6H_6O_3$	126	2.702	2.605		Sweetener
2	3-Octene-2,5-dione, 6,6,7-trimethyl-, (E)-	$C1_1H_{18}O_2$	182	2.919	0.140		preservative
3	Glutaric acid, dodec-2-en-1-yl dec-9-enyl ester	$C_{27}H_{48}O_4$	436	2.976	0.173		Sweetener/ flavouring agent
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	3.119	6.547		Flavourant
5	Benzoic acid	$C_7H_6O_2$	122	3.290	1.369		Preservative
6	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	$C_6H_6O_4$	142	3.370	0.276		Flavouring agent
7	1,3,5-Benzenetriol	$C_6H_6O_3$	126	3.536	0.120		Drug agent
8	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	3.953	68.89		Flavourant
9	1,3-Benzenediol, 2-chloro-	$C_6H_5ClO_2$	144	4.433	1.888		Antioxidant
10	1-Ethyl-2-hydroxymethylimidazole	$C_6H_{10}N_2O$	126	4.725	0.486		Stimulant

11	Succinic acid, 3-methylbenzyl octyl ester	$C_{20}H_{30}O_4$	334	5.096	4.101		Food additive
12	Lactose	$C_{12}H_{22}O_{11}$	342	5.971	0.357		Sugar moiety
13	Heptanoic acid	$C_7H_{14}O_2$	130	6.011	0.262		Preservative
14	Cyclohexanepropanol, 2,2-dimethyl-6-methylene-	$C_{12}H_{22}O$	182	6.657	0.187		Degradation product of sugar
15	2-Hexenal, 2-ethyl-	$C_8H_{14}O$	126	7.211	0.143		Flavourant
16	Caffeine	$C_8H_{10}N_4O_2$	194	7.514	6.177		Stimulant
17	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.851	0.270		Fatty acid
18	Cyclopentanol, 1-(methylenecyclopropyl)-	$C_9H_{14}O$	138	8.165	4.254		Organic solvent
19	Hexadecanenitrile	$C_{16}H_{31}N$	237	8.743	0.499		Organic solvent/dye
20	1-Ethanone, 1-(5-methyl-2-furanyl)-2-(4-quinazolinylthio)-	$C_{15}H_{12}N_2O_2S$	284	8.846	0.111		Nurotransmitter agent
21	Cyclohexanol, 3-(aminomethyl)-3,5,5-trimethyl-	$C_{10}H_{21}NO$	171	9.943	0.522		Millard product of sugar degradation
22	Protocatechoic acid, 3TBDMS derivative	$C_{25}H_{48}O_4Si_3$	496	11.052	0.262		Preservative
23	1-Hexacosanol, TMS derivative	$C_{29}H_{62}OSi$	454	11.978	0.114		Thickening agent

24	3-((1-[5-Benzyl-1-(4-fluorophenyl)-6-oxo-1,4,5,6-tetrahydro-[1,2,4]triazin-3-yl]ethylidene) hydrazone)-1,3 -dihydroindol-2-one	$C_{26}H_{21}FN_6O_2$	468	12.360	0.133		Nurotransmitter agent/ strength enhancer
25	3,5-Dihydroxybenzoic acid, 3TBDMS derivative	$C_{25}H_{48}O_4Si_3$	496	17.904	0.113		Preservative

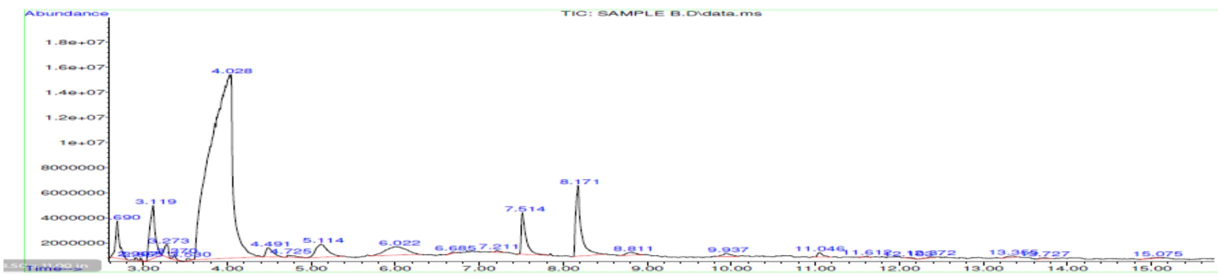
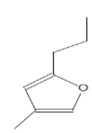
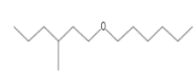
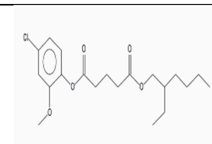
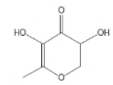
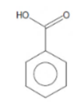
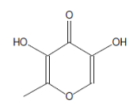
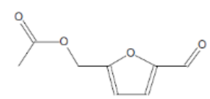
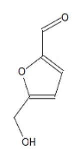
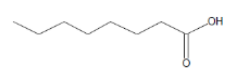
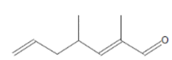
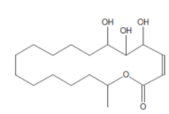
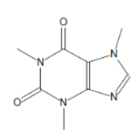
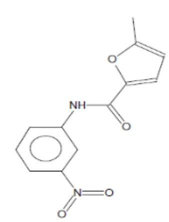


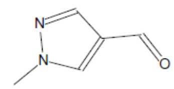
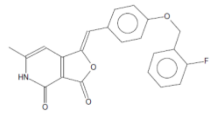
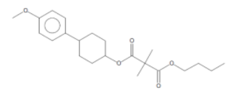
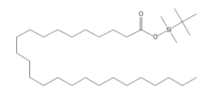
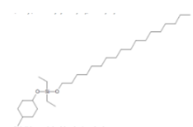
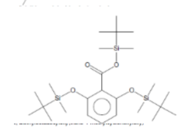
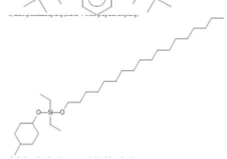
Fig. 2. GC-MS profile of organic compounds identified in samples B

Table 4, reveals the presence of the following compounds 4-methyl-2- propyl –Furan, Hexane, 1-(hexyloxy)-3-methyl-, Glutaric acid, 2- ethylhexyl 4-chloro-2- methoxyphenyl ester, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Benzoic acid, 4H-Pyran-4-one, 3,5- dihydroxy-2-methyl- and others with their percentage ion concentration.

Table 4: Components detected in sample B

S/N	Name of Compound	Mol. Formular	Mol. Wt (g)	RT (min)	% TIC	Structure	Activity
1	Furan, 4-methyl-2-propyl-	$C_8H_{12}O$	124	2.690	2.541		Sweetener
2	Hexane, 1-(hexyloxy)-3-methyl-	$C_{13}H_{28}O$	200	2.907	0.090		Flavoring agent

3	Glutaric acid, 2-ethylhexyl 4-chloro-2-methoxyphenyl ester	$C_{20}H_{29}ClO_5$	384	2.965	0.082		Flavourant
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	3.119	4.945		Flavourant
5	Benzoic acid	$C_7H_6O_2$	122	3.273	0.876		Preservative
6	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	$C_6H_6O_4$	142	3.370	0.210		Flavourant
7	5-Acetoxymethyl-2-furaldehyde	$C_8H_8O_4$	168	3.530	0.156		Flavouring agent
8	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	4.028	71.627		Flavoring agent
9	Octanoic acid	$C_8H_{16}O_2$	144	4.491	1.152		Preservative
10	2,4-dimethyl-2,6-Heptadienal	$C_9H_{14}O$	138	4.725	0.288		Sugar moiety
11	5,6,7-trihydroxy-18-methyl-Oxacyclooctadec-3-en-2-one	$C_{18}H_{32}O_5$	328	5.114	2.711		Antioxidant
12	Caffeine	$C_8H_{10}N_4O_2$	194	6.022	3.075		Stimulant
13	5-Methyl-N-(3-nitrophenyl)-2-furamide	$C_{12}H_{10}N_2O_4$	246	6.685	0.098		Antioxidant

14	Pyrazole-4-carboxaldehyde, 1-methyl-	$C_5H_6N_2O$	110	7.211	0.128		Stimulant
15	1-[4-(2-Fluoro-benzyloxy)-benzylidene]-6-methyl-1H,5H-furo[3,4-c]pyridine-3,4-dione	$C_{22}H_{16}FNO_4$	377	7.514	3.339		Neurotransmitter / strength enhancer
16	Dimethylmalonic acid, butyl 4-(4-methoxyphenyl)cyclohexyl ester	$C_{22}H_{32}O_5$	376	8.171	6.580		Antibacterial agent
17	Pentacosanoic acid, tert-butyldimethylsilyl ester	$C_{31}H_{64}O_2Si$	496	8.811	0.478		Preservative
18	1-Hexacosanol, TMS derivative	$C_{29}H_{62}OSi$	454	9.937	0.456		Antioxidant
19	Silane, diethyl(cis-4-methylcyclohexyloxy)octadecyloxy-	$C_{29}H_{60}O_2Si$	468	11.046	0.433		Thicking agent
20	2,6-Dihydroxybenzoic acid, 3TBDMS derivative	$C_{25}H_{48}O_4Si_3$	496	11.612	0.094		Preservative
21	Silane,diethyloctadecyloxy(trans-4-methylcyclohexyloxy)	$C_{29}H_{60}O_2Si$	468	12.372	0.224		Thickening agent

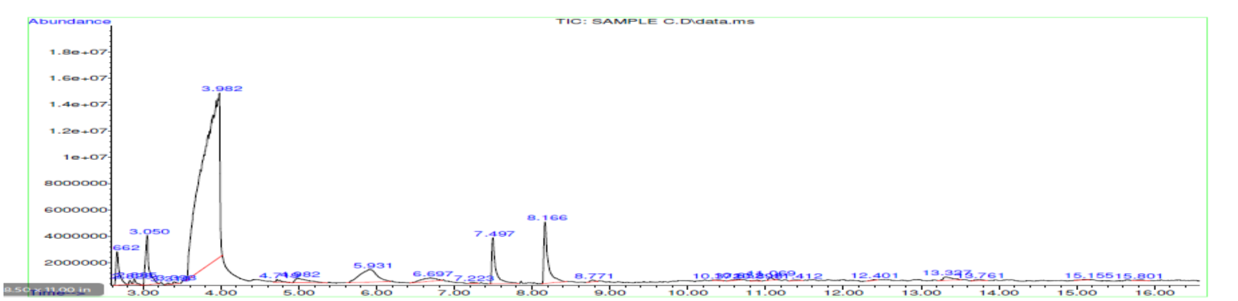
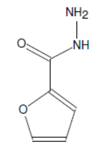
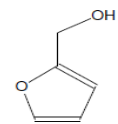
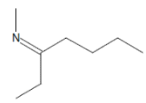
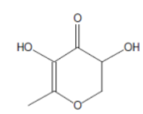
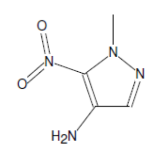
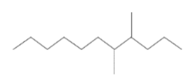
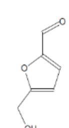
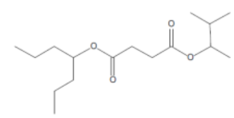
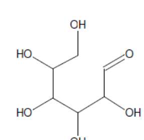
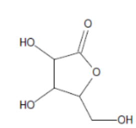
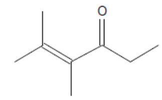
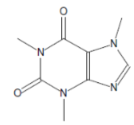
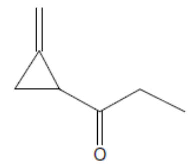
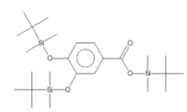
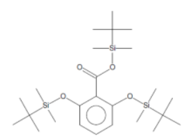
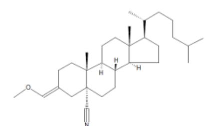
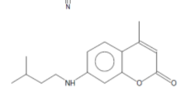
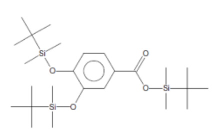
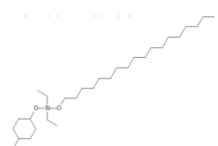


Fig. 3: GC-MS profile of organic compounds identified in samples C

Table 5, reveals the presence of the following compounds : 2-Furancarboxylic acid, hydrazide, 2-Furanmethanol, Methylamine, N-(1-ethylpentylidene)-, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 4-Amino-1-methyl-5-nitropyrazole, Undecane, 4,5- dimethyl-, 5-Hydroxymethyl Furfural, Succinic acid, 3-methylbut-2-yl 4- heptyl ester, D-Allose and other.

Table 5: Components detected in sample C

S/N	Name of Compound	Mol. Formular	Mol. Wt (g)	RT (min)	% TIC	Structure	Activity
1	2-Furancarboxylic acid, hydrazide	$C_5H_6N_2O_2$	126	2.662	2.623		Stimulant/ preservative
2	2-Furanmethanol	$C_5H_6O_2$	98	2.828	0.170		Sugar moiety/flavour ant
3	Methylamine, N-(1-ethylpentylidene)-	$C_8H_{17}N$	127	2.885	0.327		Organic solvent
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	3.050	4.198		Flavourant
5	4-Amino-1-methyl-5-nitropyrazole	$C_4H_6N_4O_2$	142	3.319	0.108		Stimulant
6	Undecane, 4,5-dimethyl-	$C_{13}H_{28}$	184	3.393	0.162		Organic solvent
7	5-Hydroxymethyl Furfural	$C_6H_6O_3$	126	3.982	71.68 8		Flavourant
8	Succinic acid, 3-methylbut-2-yl 4-heptyl ester	$C_{16}H_{30}O_4$	286	4.719	0.318		Antioxidant
9	D-Allose	$C_6H_{12}O_6$	180	4.982	1.395		Simple sugar
10	D-(+)-Ribonic acid γ-lactone	$C_5H_8O_5$	148	5.931	5.211		Flavouring agent

11	4-Hexen-3-one, 4,5-dimethyl-	$C_8H_{14}O$	126	6.697	1.213		Flavouring agent
12	Caffeine	$C_8H_{10}N_4O_2$	194	7.223	0.108		Stimulant
13	Cyclopropane, 2-methylene-1-propionyl-	$C_7H_{10}O$	110	7.497	4.278		Organic solvent
14	1-Hexacosanol, TMS derivative-	$C_{29}H_{62}OSi$	454	8.166	6.066		Anti diabetic agent
15	Protocatechoic acid, 3TBDMS derivative	$C_{25}H_{48}O_4Si_3$	196	8.771	0.205		Preservative
16	2,6-Dihydroxybenzoic acid, 3TBDMS derivative	$C_{25}H_{48}O_4Si_3$	496	10.372	0.083		Preservative
17	5Alpha-cyano-3-methoxymethylenecholestane, (E)-	$C_{30}H_{49}NO$	439	10.652	0.151		Steroid for strength enhancement
18	7-(3-Methylbutyl)amino-4-methylcoumarin	$C_{15}H_{19}NO_2$	245	10.898	0.091		Antibacterial agent
19	Protocatechoic acid, 3TBDMS derivative	$C_{25}H_{48}O_4Si_3$	496	11.069	0.122		Preservative
20	Silane, diethyloctadecyloxy(trans-4-methylcyclohexyloxy)-	$C_{29}H_{60}O_2Si$	468	11.412	0.104		Chemical adhesive/ thickener

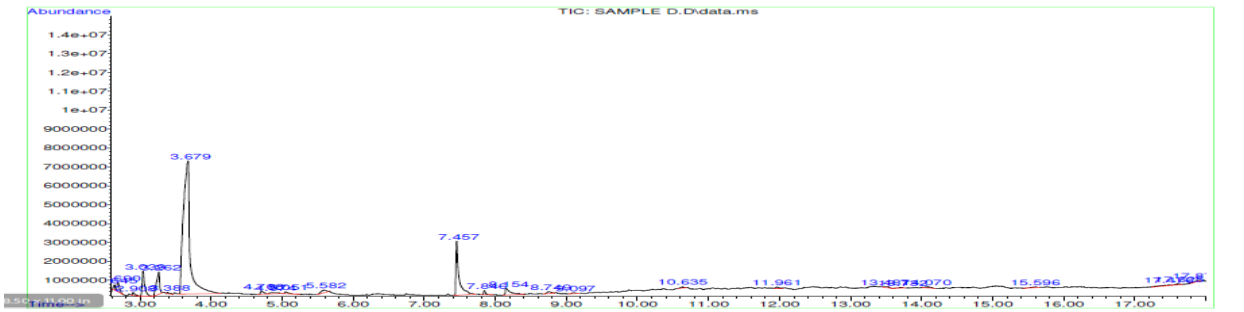
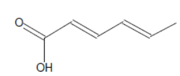
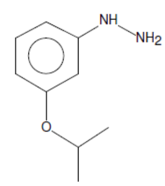
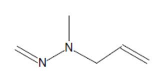
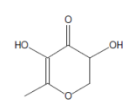
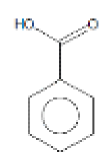
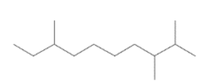
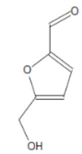
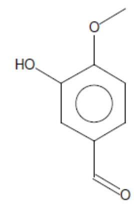
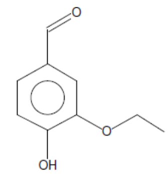
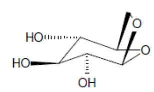
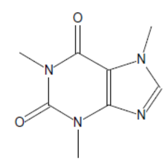
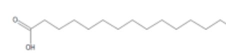
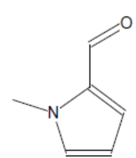
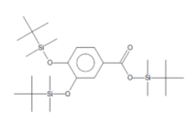
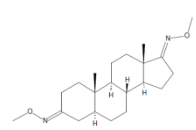
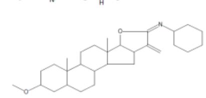


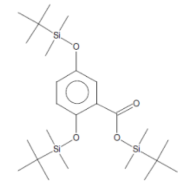
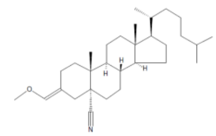
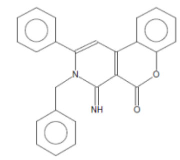
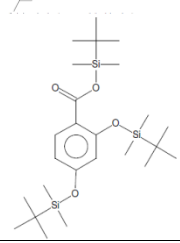
Fig. 4: GC-MS profile of organic compounds identified in sample D

Table 6, reveals the presence of the following compounds: Sorbic Acid, 3-isopropoxy-, Phenylhydrazine, Formaldehyde, methyl(2-propenyl)hydrazone, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Benzoic acid, 2,3,8-trimethyl- Decane, 5-Hydroxymethylfurfural, Benzaldehyde, 3-hydroxy-4-methoxy-, Ethyl Vanillin and others.

Table 6: Components detected in sample D

S/N	Name of Compound	Mol. Formular	Mol. Wt (g)	RT (min)	% TIC	Structure	Activity
1	Sorbic Acid	$C_6H_8O_2$	112	2.645	0.555		Anti fungi
2	3-isopropoxy-Phenylhydrazine	$C_9H_{14}N_2O$	166	2.690	1.565		Phenylhydrazones (separate simple sugars)
3	Formaldehyde, methyl(2-propenyl)hydrazone	$C_5H_{10}N_2$	98	2.908	0.483		Food preservative
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	3.039	4.609		Flavouring agent / antioxidant
5	Benzoic acid	$C_7H_6O_2$	122	3.262	5.118		preservative
6	2,3,8-trimethyl- Decane	$C_{13}H_{28}$	184	3.388	0.238		Organic solvent

7	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	3.679	65.136		Flavouring agent/ food additive
8	Benzaldehyde, 3-hydroxy-4-methoxy-	$C_8H_8O_3$	152	4.708	0.776		Flavourant
9	Ethyl Vanillin	$C_9H_{10}O_3$	166	4.874	0.673		Flavourant
10	1,6-anhydro-β-D-Glucopyranose	$C_6H_{10}O_5$	162	5.051	0.536		Product of sugar degradation
11	Caffeine	$C_8H_{10}N_4O_2$	194	5.582	1.123		Stimulant
12	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	11.923	11.923		Organic solvent
13	1H-Pyrrole-2-carboxaldehyde, 1-methyl-	C_6H_7NO	109	7.846	0.653		Millard reaction by product
14	Protocatechoic acid, 3TBDMS derivative	$C_{25}H_{48}O_4Si_3$	496	8.154	2.453		Antioxidant
15	Androstane-3,17-dione, bis(O-methyloxime), (5α)-	$C_{21}H_{34}N_2O_2$	346	8.749	0.603		Steroid for muscular /strength
16	Androsta[17-16-b]furan-5'-imine, 4'-methylene-3-methoxy-N-cyclohexyl-	$C_{29}H_{45}NO_2$	439	9.097	0.233		Steroid for muscle strength

17	2,5-Dihydroxybenzoic acid, 3TBDMS derivative	$C_{25}H_{48}O_4Si_3$	496	10.635	0.314		Preservative
18	5Alpha-cyano-3-methoxymethylenecholestane, (E)-	$C_{30}H_{49}NO$	439	11.961	0.295		strength enhancement
19	5H-[1]Benzopyrano[3,4-c]pyridin-5-one, 3,4-dihydro-4-imino-2-phenyl-3-(phenylmethyl)--	$C_{25}H_{18}N_2O_2$	378	13.487	0.280		Neurotransmitter Agents
20	Silane, diethyloctadecyloxy(trans-4-methylcyclohexyloxy)-	$C_{29}H_{60}O_2Si$	468	14.070	0.450		Chemical adhesive/ thickener
21	2,4-Dihydroxybenzoic acid, 3TBDMS derivative	$C_{25}H_{48}O_4Si_3$	496	15.596	0.318		Perseveration

Spectral data analysis of organic compounds

This study revealed that about 70 % of compounds identified are degradation product of starch and there were in agreement with the report of Maurice [32]. The compounds identified include: Furyl hydroxymethyl ketone with percentage total ion concentration (%tic) of 2.606% and molecular formula of $C_6H_6O_3$, molecular weight 126, Lactose (%tic) of 0.375% and molecular formula of $C_{12}H_{22}O_{11}$; 4-methyl-2-propyl-Furan(2.542%), and molecular formula $C_8H_{12}O$; 5-Acetoxymethyl-2-furaldehyde (0.156%) and molecular formula $C_8H_8O_4$; 5-Hydroxymethyl furfural (71.627%) and formula $C_6H_6O_3$. 2,6-Heptadienal, 2,4-dimethyl- (0.288%) molecular formula $C_9H_{14}O$. Others are: 2-Furancarboxylic acid, hydrazide (2.623%) and molecular formula $C_5H_6N_2O_2$; 2-Furanmethanol (0.170%), molecular formula $C_5H_6O_2$ molecular weight 98. D-(+)-Ribonic acid γ-lactone (5.211%), molecular formula and weight $C_5H_8O_5$ and 148 respectively. D-Allose (1.395%), molecular formula $C_6H_{12}O_6$, and weight 180 in all the samples analyzed. These implies that they are made mostly from natural products. Xylans and glucans can be transformation into furfural under mild processing conditions. However, recent technique involves the reactions of simple pentoses or pentosans, which offers significant advancements in the valorization of native carbohydrates into furfural [33].

Research has also demonstrated that the acid-catalyzed conversion of hexose sugars (such as fructose and glucose) to furans results in the production of furyl hydroxymethyl ketone. Nonetheless, the following substances that have been identified elsewhere as stimulants were found in all four energy drink samples that were examined, along with their percentage concentration: In samples A, B, and C, the caffeine content were 6.177 %, 3.075%, and 0.108 %, respectively, whereas in sample D, the caffeine content was 1.123%. According to Jones [34] caffeine is a stimulant that significantly affects the central nervous system. Compared to caffeinated beverages that contain 50–100 mg of caffeine, energy drinks typically contain 70–200 mg of caffeine, making it the most prevalent stimulant [35]. When taken moderately (3-6 mg/kg of body mass) before and/or during exercise, caffeine has been demonstrated to be an effective ergogenic aid for endurance athletes; the effect will be maximized if caffeine is avoided for at least 7 days prior to use [36, 37].

Nonetheless, the International Olympic Committee has listed caffeine as one of the prohibited substances [38]. The diuretic effect of caffeine may be diminished if the body rapidly develops a tolerance to it [39. 40**].** According to recent studies, long-term caffeine users can overcome the mild diuretic effects of caffeine and maintain appropriate water levels [41, 42]. Other compounds of interest in all the four Energy drinks studied include: 5-Hydroxymethylfurfural, a flavourant principle with percentage total ion concentration of (68.890%) in sample A, 71.627% in sample B, (71.688%) in sample C and 65.136% in sample D. It is important to note that all the four energy drink samples analyzed has a very high percentage content of 5-hydroxymethylfurfural, a dehydration product of reducing sugars. Also the samples revealed the presence of Glutaric acid, dodec-2-en-1-yl dec-9-enyl ester (0.173%) and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (6.547%) flavourant principles in samples A and B, while sample C and D depicts D-Allose (1.395%), D-(+)-Ribonic acid γ-lactone (5.211%) and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (4.609%), Ethyl Vanillin (0.673%) and 1,6-anhydro-β-D-Glucopyranose, (0.536%) in samples C and D respectively. Steroidal compounds identified with their percentage ion concentration include: 5Alpha-cyano-3-methoxymethylenecholestane, (E)- (0.151%), Androstane-3,17-dione, bis(O-methyloxime), (5α)- (0.603%), Androsta[17-16-b] furan-5-imine, 4-methylene-3-methoxy-N-cyclohexyl- (0.233%) and 5Alpha-cyano-3-methoxymethylenecholestane, (E)- (0.295%). Anabolic steroids are hormones that promote muscle growth and increase strength and energy. However, they can also have many side effects, including psychologic (mood swings, aggressive behavior, irritability) and physical (acne, masculinizing effects in women, breast enlargement in men) [43].

Conclusion

Energy drinks are widely recognized for their ability to improve physical fitness and prevent obesity. Numerous supplements that claim to improve performance, muscle growth, and recovery have emerged as a result of this trend. However, the safety of energy drinks has not been thoroughly determined, and prolonged exposure to the different ingredients in energy drinks may cause major changes in the cardiovascular system. The physicochemical analysis results in this study showed that some parameters were found to be above limits, while the essential metal ion concentration results were found to be within the FDA-permissible limits. The metabolic profiling, on the other hand, showed that caffeine was present in all four of the energy drink samples that were examined.

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