

## GC-MS AND HEAVY METAL ANALYSIS OF GASCA D HERBAL FORMULATION

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**Abstract**

Gasca D herbal formulation is an antidiabetic medicine for the management of diabetes mellitus produced exclusively from natural ingredients. The level of some essential elements (Copper, chromium, Cobalt, Iron, Manganese, Nickel and zinc) and toxic (Cadmium, Arsenic, Mercury, and Lead) heavy metals were determined using microwave plasma-atomic emission spectrometry (MP-AES). The aim of this study is to evaluate the levels of essential and toxic heavy metals and also identify bioactive compounds present in Gasca D herbal formulation. The result shows no traces of Lead, Mercury, Zinc, Arsenic and Zinc, Iron was found to have highest concentration  $67.16 \pm 7.5 \mu\text{g/g}$  and Cadmium lowest concentration  $0.4 \pm 0.03 \mu\text{g/g}$ . GC-MS analysis of Gasca D herbal formulation revealed the presence of 14 biologically active compounds which include N-Formyl-beta-alanine, Paromomycin, 3,4-Altrosan, Benzamide, 1,3,4-Thiadiazol-2-amine, Carbamodithioic acid, Carbonic acid, alpha-D-Glucopyranoside, Ethyl isocyanide, 2-Propanesulfinic acid, Propanamide, 2-Butenenitrile, Dicyclopropyl carbinol, Isoxazolidine, 1,5-Hexadiene 10-Azido-1-decanethiol. *Conclusion:* The result indicates that the mean levels of toxic metals in Gasca D herbal formulation were below WHO permissible levels. Gasca D herbal formulation also contains various bioactive compounds that can have various medicinal application which can be used for the treatment of various diseases.

## Introduction

In all countries of the world there exists traditional knowledge related to the health of humans and animals. In Africa, traditional healers and remedies made from plants play an important role in the health of millions of people. In Africa there is wide disparity between the relative ratios of traditional practitioners and university trained doctors in relation to the whole population. This may be reason why almost eighty per cent of African populations use some form of traditional herbal medicine [1,2].

With the ever-increasing use of herbal medicines worldwide, the safety and quality of medicinal plant materials and finished herbal medicinal products have become a major concern for health authorities, pharmaceutical industries and the public. It is estimated that up to four billion people (representing 80% of the world's population) living in the developing world rely on herbal medicinal products as a primary source of healthcare and traditional medical practice which involves the use of herbs is viewed as an integral part of the culture in those communities [3-5].

As the global use of herbal medicinal products continues to grow and many more new products are introduced into the market, public health issues, and concerns surrounding their safety are also increasingly recognized. Although some herbal medicines have promising potential and are widely used, many of them remain untested and their use also not monitored. This makes knowledge of their potential adverse effects very limited and identification of the safest and most effective therapies as well as the promotion of their rational use more difficult [1].

It has become necessary to investigate herbal medicinal products to facilitate better understanding of the risks associated with the use of these products and to ensure that all medicines are safe and of suitable quality.

Medicinal plants, unlike pharmacological drugs, commonly have several chemicals working together catalytically and synergistically to produce a combined effect that surpasses the total activity of the individual constituents. The combined actions of these substances tend to increase the activity of the main medicinal constituent by speeding up or slowing down its assimilation in the body. Secondary metabolites from plant's origins might increase the stability of the active compound(s) or phytochemicals, minimize the rate of undesired adverse side effects, and have an additive, potentiating, or antagonistic effect.

However the safety levels of these herbal remedies are not fully investigated. Most herbal medicines are not subjected to rigorous scrutiny in terms of safety and efficacy as is the case for conventional western medicine, and heavy metals have been reported in some of these herbal products [6,7]. These heavy metals constitute health risk to users because they bioaccumulate in the body when ingested via fluids, food or through other sources of contamination and are stored faster than they are broken down or excreted [7,8]. These shows that there is urgent need to investigate in order to determine the concentration of the some toxic metals which pose significant health risk to people. The need for safety assessment and quality assurance of plant products has been highlighted by the WHO. It seems compulsory that all herbal preparations and raw materials be investigated for the presence of heavy metals [9].

The main aim of this study is to quantify the level of some selected toxic metals such as arsenic, cadmium, lead and mercury in Gasca D herbal products manufactured by Greenleaf herbal product in Kano, Nigeria.

## MATERIALS AND METHODS

**Reagents:** Analytical grade nitric acid, hydrochloric acid (Sigma aldrich).

### Digestion of the samples

Gasca D herbal sample with mass 5g was weighed and dissolved in 10mL of mixed acid (HNO<sub>3</sub>/HCl in the ratio 1:3) for 12hr. The mixture was heated on a hot plate until the brown fumes changed to white. It was allowed to cool and 10 mL of distilled water was added and again heated again. The mixture was then filtered into 50 mL volumetric flask after cooling. The final volume was made up to 50 mL with deionized water. Reagent blank was also carried out in the same way. All measurements were done in duplicate for the sample and standard solution.

### Instrumentation

Agilent 4100 MP-AES was used for the total metal determination of Zn, Cu, Pb, Fe, Mo, Hg, Si, V, Cd, Ni, Co, Mn, Cr, Al and As in Gasca D herbal formulation. The viewing position and nebulizer pressures were optimized automatically using the Agilent MP Expert software. Manual sample introduction mode was used. Table 1 lists the Agilent 4100 MP-AES operating conditions

**Table 1: Agilent 4100 MP-AES operating conditions**

Instrument parameter	Setting
Nebulizer	Concentric
Spray chamber	Single-pass glass cyclonic
Sample tubing	Black/black
Waste tubing	Blue/blue
Read time	3s
Number of replicates	2
Stabilization time	10s
Fast pump during sample uptake	On
Pump speed	15 rpm

### Calibration

Analytical calibration was carried out using multi-element standard solutions, except for As. Separate calibration solutions were used for As because of the Fe spectral interference on the As 234.984 nm emission line.

### Background and interference corrections

The auto-background correction feature in the MP Expert software was used for background correction. Fast Linear Interference Correction (FLIC), an Agilent proprietary spectral interference correction method, was used to correct and remove the iron interference on determination of As using the 234.984 nm line.

### Gas chromatography-mass spectrometry (GC-MS) analysis

The Gasca D herbal formulation powdered sample was dissolved in HPLC grade methanol to appropriate concentrations for GC-MS analysis, and was analyzed by GC-MS on an Agilent 6890N series gas chromatograph coupled with a LECO time of flight mass spectrometer detector (MS-TOF; Agilent Technology, Santa Clara, CA) with ionization voltage of 70 eV and equipped with fused silica non-polar DB-5 capillary column (10m x 0.18 mm; film thickness 0.18 mm; Agilent HP). The operating conditions were as follows: the injector operated in split mode (ratio

20:1) and mass spectrometer transfer line temperatures were set at 200 and 300 °C, respectively; the oven temperature gradually rose from 40 to 260 °C, at warming rate of 4 °C min<sup>-1</sup>, kept at 260 °C during 20 min, and finally up to 340 °C, at a warming rate of 4 °C min<sup>-1</sup> for 20 min isothermally; the injector temperature was set at 300 °C. The carrier gas (He) was set to 1mL min<sup>-1</sup> flow. Mass analyzer was used in full scan mode scanning from m/z 40-550 and mass spectra were taken at 70 eV. Compounds were identified by coinjection of the sample with standard references by comparison of their mass spectral data with those of NIST Mass Spectral Library 98 and equipment Libraries as well as by comparison of their retention indices (RIs).

## RESULTS

The element with the highest concentration found in Gasca D herbal formulation is iron 67.16 µg/g, followed by aluminium with a concentration of 16.84µg/g. The concentrations of Lead, Zinc, Mercury, Silicon, Arsenic and Molybdenum were not detected at all; the values were 0.0µg/g, 0.0µg/g, 0.0µg/g, 0.0µg/g, 0.0µg/g and 0.0µg/g respectively (Table 2). Cadmium and Chromium were found in trace amount at a concentration of 0.4µg/g and 0.72µg/g respectively. Other elements found in Gasca D herbal formulations are Cu, V, Ni, Co and Mn with relative concentrations of 1.6 µg/g, 2.6 µg/g, 1.72 µg/g, 1.64 µg/g and 7.72 µg/g respectively (Table 2).

**Table 2: Result for the analysis of Gasca D**

Analyte	MP-AES results Average ± SD (µg/g)
Zn	0 ± 0
Cu	1.6 ± 0.4
Pb	0 ± 0
Fe	67.16 ± 7.5
Mo	0 ± 0
Hg	0 ± 0
Si	0 ± 0
V	2.6 ± 0.7
Cd	0.4 ± 0.03
Ni	1.72 ± 0.05
Co	1.64 ± 0.1
Mn	7.72 ± 0.9
Cr	0.72 ± 0.04
Al	16.84 ± 1.7
As	0 ± 0

### GC-MS analysis of compounds identified in Gasca D herbal formulation

The GC-MS analysis of Gasca D herbal formulation revealed the presence 14 main compounds. The compounds are listed in (Table 3); shown are the phytochemicals retention time, molecular formula

and molecular weight (MW). The examination of Gasca D herbal formulation allowed the identification of a very important antibiotic paromomycin and Thiadiazol derivative which is a new class of carbonic anhydrase inhibitors, the herbal formulation was found to have higher amounts of carbonic acid appearing at different peak interval, derivatives of amide, nitrile were also identified (Table 3).

**Table 3: Phytocomponents identified in the Gasca D herbal formulation by GC-MS analysis**

S/N	Compound Name	PEAK	R.TIME (s)	M. Formula	M.Wt. (g/mol)
1	1,5-Hexadiene	7	58.8	C <sub>6</sub> H <sub>10</sub>	82.143
2	2-Propanesulfinic acid	10	60.6	C <sub>3</sub> H <sub>8</sub> O <sub>2</sub> S	108.161
3	Propanamide	16	64.5	C <sub>3</sub> H <sub>7</sub> NO	73.095
4	2-Butenenitrile	18	66.4	C <sub>4</sub> H <sub>5</sub> N	67.091
5	Dicyclopropyl carbinol	19	67.0	C <sub>7</sub> H <sub>12</sub> O	112.1696
6	Isoxazolidine, 10-Azido-1-decanethiol	21	69.2	C <sub>3</sub> H <sub>7</sub> NO C <sub>10</sub> H <sub>21</sub> N <sub>3</sub> S	73.095 215.359
7	Ethyl isocyanide	23	70.8	C <sub>3</sub> H <sub>5</sub> N	55.08
8	alpha-D-Glucopyranoside	27	74.1	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	180.156
9	Carbonic acid	30;32;33;40	75.8; 76.4;76.7;80.4	CH <sub>2</sub> O <sub>3</sub>	62.024
10	Carbamodithioic acid	31	76.0	C <sub>3</sub> H <sub>7</sub> N S <sub>2</sub>	121.224
11	1,3,4-Thiadiazol-2-amine	36	77.8	C <sub>2</sub> H <sub>3</sub> N <sub>3</sub> S	101.127
12	Paromomycin 3,4-Altrosan	38	78.8	C <sub>23</sub> H <sub>45</sub> N <sub>5</sub> O <sub>14</sub>	615.634
13	Benzamide	44;47	85.8	C <sub>7</sub> H <sub>7</sub> NO	121.139
14	N-Formyl-beta-alanine	20	68.309	C <sub>4</sub> H <sub>7</sub> NO	117.103

## DISCUSSION

It is well known that heavy metals in herbal products pose a substantial health hazard due to their toxic and mutagenic effects, regardless of the concentration levels [10]. Herbs are taken in different forms as teas, syrup, essential oils, capsules and tablets. The world health organization has mandated all medicinal plants which form the raw material for the finished product must be checked for the presence of heavy metal and pesticide residues [11]. Result of the present study showed no traces of Lead, Mercury, Silicon, Arsenic, Molybdenum and Zinc (Table 2). The lack of any detectable amount of the above metals in Gasca D herbal formulation is an indication that the Gasca D herbal formulation may not pose serious health hazard to human health, as most of these heavy metals are found to be toxic in high concentration to most of the major organs such as kidney, liver, renal tract, cardiovascular, reproductive and others [12]. This shows that the formulation can be safe for consumption.

Toxicity of Cadmium can occur if their concentration is found to be above the permissible limit set by WHO, China and Thailand as 0.3  $\mu\text{g/g}$  in medicinal herbs [13]. The level of Cadmium found in the present study was 0.4 $\mu\text{g/g}$  which is much lower than the permissible limit of 30 $\mu\text{g/g}$  [13]. Gasca D herbal formulation was found to have Cd level lower than most herbal formulation being sold in Nigeria. Chionyedua et al. [14] reported the level of Cd in some popular registered herbal formulation sold in Nigeria such as E-5000, YC-BITTER and B-CAPS had Cd concentration of 0.48 $\mu\text{g/g}$ , 0.52 $\mu\text{g/g}$  and 3.08 $\mu\text{g/g}$  respectively. The calculated daily limit of Canada natural product daily limit for cadmium is 8.5 $\mu\text{g/g}$  [15]. This shows that the cadmium level of Gasca D herbal formulation is far below the maximum daily limit set by Canada, and it may thus not pose any significant health risk. Chromium is an essential micronutrient, but it becomes toxic at high levels, and chronic exposure to Cr may result in liver, kidney and lung damage [16]. The level of chromium found in the present study was 0.72 $\mu\text{g/g}$  which is lower than the tolerable upper intake level of 30 $\mu\text{g/day}$  [17]. The permissible limit for chromium in herbal medicinal plants/finished products have not been set by the World Health Organization but the Cr level in Gasca D herbal formulation may not pose serious health effect to humans. The Cu, Ni, and Mn content of Gasca D herbal formulation with relative concentrations of 1.6 $\mu\text{g/g}$ , 1.72 $\mu\text{g/g}$ , and 7.72 $\mu\text{g/g}$  respectively were all found to be below the concentration of many herbal formulations and also below the tolerable upper intake level of Cu, 900 $\mu\text{g/g}$ , Ni, 1000  $\mu\text{g/g}$  and Mn, 2000  $\mu\text{g/g}$  respectively as reported by Bogdan and Marek, [17]. The cobalt concentration of 1.64 $\mu\text{g/g}$  obtained in this work is lower than the one reported by Jabeen *et al.* [18], in which seven herbal medicines used in Turkey had (0.14 mg/kg-0.48 mg/kg).

Vanadium an element of the transition metals is present in the air and soil contaminants in large urban agglomerations due to combustion of fossil fuels. It forms numerous inorganic compounds (vanadyl sulfate, sodium metavanadate, sodium orthovanadate, vanadium pentoxide) as well as complexes with organic compounds (BMOV, BEOV, METVAN). Vanadium concentration of Gasca D herbal formulation was found to be 2.6 $\mu\text{g/g}$ . At ambient conditions, the daily oral intake of vanadium via potable water and food varies between 10  $\mu\text{g}$  and 2 mg. This is clearly beyond the no-effect level of 10 mg vanadium per day and per kg body mass [19]. Vanadium compounds lower blood glucose levels in animals and in clinical trials. They also inhibit the activity of protein tyrosine phosphatase 1B. Vanadium compounds, in particular organic derivatives, have insulin-mimetic and antidiabetic properties. The presence of Vanadium in Gasca D herbal formulation is evidence that it might have played a significant role in reduction of blood glucose concentration found in Alloxan induced diabetic rats that received Gasca D at 1,000mg/kg bw. Therapeutic properties of vanadium compounds in diabetes appeared as early as 1899 [20]. Studies performed in diabetic patients, confirmed the therapeutic effect of vanadium compounds on blood glucose



levels with little side effects [21]. And the mechanism of reduction of blood glucose levels by vanadium compounds has been reported to be via the activation of PKB/Akt leading to increased uptake of glucose by the GLUT4 transporter [22].

Gasca D herbal formulation has been found in the present study to have Aluminium at a concentration of 16.84 $\mu$ g/g. The mean exposures of the adult population to aluminium from overall diet including additives varied a lot among different countries, ranged from 1.6 mg/day in most recent French study to more than 34 mg/day [23] in Mainland China [24]. In fact, the dietary exposures to aluminium of some population groups were found to exceed the Provisional Tolerable Weekly Intake PTWI in some countries such as the UK (1.3 mg/kg bw/week for toddlers (1.5 – 4.5 years)) [15], Sweden (1.5 mg/kg bw/week for 60-kg females)<sup>2</sup>, and Mainland China (4.0 mg/kg bw/week for 60-kg adults) [24]. Aluminium is present in drinking water at usual levels of less than 0.2 mg/L, and is also present in most foods naturally (normally at levels of less than 5 mg/kg). But, some foods such as potatoes, spinach and tea may contain high levels of aluminium naturally. Aluminium is also present in food owing to the use of aluminium-containing food additives, which has been regarded as the main dietary source [25,26]. Moreover, it has been reported that soya-based formulae were found to contain high levels of aluminium, leading to concentrations of 0.4 – 6 mg/L in the ready-to-drink products [26]. No acute toxic effects by the oral exposure to aluminium in the general population have been reported despite its widespread occurrence in foods, drinking water and many antacid preparations [27]. Daily intake of aluminium in antacids and buffered aspirin could be as much as 5 g and 0.7 g respectively [26]. For an individual who regularly ingests aluminium-containing medications for long term, exposure to aluminium from medication could be much higher than that from the diet. The concentration of iron in the Gasca D herbal formulation was the highest among all the elements tested with a concentration of 67.16 $\mu$ g/g. Iron is an essential element that is necessarily required for boosting the red blood cell. The presence of iron may play a significant role to protect, maintain and enhance red blood cell function.

GC-MS analysis of Gasca D herbal formulation shows the presence of 14 important compounds. One of such compounds is beta-alanine, which has been shown to enhance muscular endurance. Beta-alanine supplementation is used by athletes competing in high-intensity track and field cycling, rowing, swimming events and other competitions [28,29]. The presence of beta-alanine in Gasca D herbal formulation may be very helpful especially for people with type 2 diabetes who have developed keto acidosis, lactic acidosis due to medication or those who suffer from fatigue, malaise and lack of vigor. Beta-alanine supplementation increases intramuscular L-carnosine concentrations, combining with muscle L-histidine [30]. During moderate to high-intensity exercise, hydrogen ions (H<sup>+</sup>) begin to accumulate leading to a drop in intramuscular pH and ultimately influencing muscle performance [31]. Carnosine has been shown to act as an intracellular buffer to Hydrogen [32], which may help the muscle 'buffer' or withstand the fatigue more effectively. One such compound with buffering capacity found in Gasca D herbal formulation is carbonic acid, which appeared at four different retention time intervals signifying the abundance of this compound in the herbal mixture. Carbonic acid is naturally present in the human body to maintain a normal pH balance in the blood. Carbonic acid as with most buffers in the human body can be exhausted, they need to be replaced. One of the most common uses of carbonic acid is as a salt form, sodium bicarbonate, for an intravenous infusion in the hospital setting. It is the main buffering compound in human blood and can be broken down to carbon dioxide by an enzyme called carbonic anhydrase. Another compound that has been found to

influence carbonic anhydrase activity in Gasca D herbal formulation is 1,3,4-Thiadiazol-2-amine. 1,3,4-Thiadiazoles were first described in 1882 by Fischer and further developed by Busch and his coworkers. Thiadiazoles carrying mercapto, hydroxy and amino substituents can exist in many tautomeric forms [33]. They are widely known as compounds with various kinds of biological activities showing anticancer properties against human cancers and acting as diuretic, antibacterial, antifungal, antitubercular and leishmanicidal agents. Many of them exhibit anticonvulsant, anti-inflammatory, antidepressant, analgesic and anxiolytic effects. The molecular target of 1,3,4-thiadiazoles includes the following enzymes: carbonic anhydrase (CA), cyclooxygenase (CO), neutral endopeptidase (NEP), aminopeptidase N (APN), matrix metalloproteinases (MMPs), phosphodiesterases (PDEs) and c-Src/Abl tyrosine kinase [34].

Paromomycin is another compound identified in Gasca D herbal formulation. Paromomycin is an aminoglycoside antibiotic produced by *Streptomyces rimosus* var. *paromomycinus* [35], that has been widely used in human to treat leishmaniasis, cryptosporidiosis, and amebiasis [36] and veterinary medicine for the treatment of various bacterial infections. The antibacterial spectrum of paromomycin is similar to other aminoglycosides that demonstrate broad spectrum activity against some gram-positive and many gram-negative bacteria [27].

## CONCLUSION

In this study the level of some toxic metals were measured and found the most commonly toxic metal to be below the detection limit or in trace amount. The study has also reported the presence of bioactive compounds with biological activity. The results of the study clearly indicate the presence of compounds with pharmacological activities that can be effectively used to treat diseases like cancer, diabetes mellitus, arthritis and inflammation. Further study such as isolation and bioassay of these derivatives should be carried out in the future.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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