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Chemical analysis of *Agama agama* Linnaeus, 1758. (Agamidae) excreta: Emerging trends in substance abuse in Nigeria

[1*]Charles G. Ikimi and [2]Onoriode A. Udi

[1]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Federal University Otuoke, Bayelsa State, Nigeria.
[2]Department of Human Anatomy, Faculty of Basic Medical Sciences, Federal University Otuoke, Bayelsa State, Nigeria.

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ABSTRACT

Background: Absence of data on the chemical composition of the excreta of *Agama agama*, a novel substance widely abused by young people in Nigeria prompted an investigation into the excreta of *A. agama* in order to identify and assess its psychoactive and toxic contents, so as to make informed decisions on its use.

Methods: Fresh excreta of *A. agama* were collected from uncompleted buildings in the Federal University, Otuoke, Bayelsa state. Collected samples were sun dried for ten days, pulverized and then processed for analysis. The chemical constituents of the concentrated extracts of *A. agama* excreta were determined with a Gas Chromatography-Mass Spectrometry (GC-MS) machine.

Results: The result revealed seventeen compounds along with their nomenclature, chemical formula, structure, molecular weight, retention time and peak area. The most abundant constituent was 9,12-Octadecadienoic acid (17.53%). The constituent with the highest retention time (20.417mins) was methyl-16-hydroxyhexadecanoate while the constituent with the highest molecular weight (334 Da) was cyclopropaneoctanoic acid. Psychoactive compounds identified among the constituents were nonanoic acid, n-hexadecanoic acid and 8-methyl-6-nonenamide while the toxic chemicals identified among the constituents were 1-eicosene, nonanoic acid, n-hexadecanoic acid, 8-methyl-6-nonenamide, 9,12-octadecadienoic acid, hexadecanoic acid, 11-octadecanoic acid, 15-hydroxypentadecanoic acid and methyl stearate.

Conclusion: The toxic compounds identified in *A. agama* excreta pose significant health risks to individuals who consume it. This study provides empirical evidence to discourage the unregulated use *A. agama* excreta for its psychoactive effects. Hence, there is serious need for policy formulations and public awareness measures to educate, sensitize and cascade these discoveries to young people.

Keywords: *Agama agama*, GC-MS, psychoactive, substance abuse, toxic.

1.0 INTRODUCTION

Substance abuse among Nigerian youths has become a significant concern across various societal levels. Globally, it has been estimated that 90% of population aged 12 years or older are diagnosed of dependence on psychoactive substances [1]. United Nations Office on Drugs and Crime (UNODC) reported that consumption of these psychoactive substances is associated with adverse health concerns. It is suspected that the abuse of such substances will lead to the risk of developing several pathologies such as mental disorder, insomnia, breathing difficulties, concentration problems and many other behavioral disorders [2]. Individuals frequently experiment with accessible materials in search of novel mood-altering substances. Most of the commonly abused biological substances are of

[*]**Corresponding author: Email:** ikimicg@fuotuoke.edu.ng; **Phone: +234 (0) 8065110063**

plant origin, such as leaves of marijuana, cannabis, *Moringa oleifera* and *Carica papaya* [1]. In recent times, reports have shown that substances of animal origin are now being abused. Amongst such substances are the excreta of the reptile *Agama agama*, and the tail of the reptile *Hemidactylus frenatus* [3]. Substance abuse is harmful or hazardous use of psychoactive substances, including alcohol and illicit drugs. A psychoactive substance is a chemical substance that acts mainly on the central nervous system where it alters brain function, thereby resulting in temporary changes in perception, mood, consciousness and behavior [4]. Substances which are both rewarding and positively reinforce their intake have the potential to induce a state of addiction which is a state of compulsive use of a substance or drug despite negative consequences [5]. Psychoactive substance use can lead to dependence syndrome - a cluster of behavioural, cognitive, and physiological phenomena that develop after repeated substance use. This typically include a strong desire to take the drug, difficulties in controlling its use, persisting in its use despite harmful consequences, a higher priority given to drug use than to other activities and obligations, increased tolerance, and sometimes a physical withdrawal state. There are over 190 million drug users around the world. Substance abuse is increasing at alarming rates, especially among young adults. Substance abuse is major social ill that is of public health importance [6], lifetime substance use among youth is sometimes higher than 50% [7] and there is increasing alarming incidence of substance abuse among the youth in the society ranging from conventional to non- conventional substance abuse. In Nigeria, there is an alarming increase in the use of cheap household chemical products and several other unconventional materials for the purpose of altering consciousness. Psychoactive substances are now prevalent among the youths in Nigeria. Youths indulge in the nonconventional use of psychoactive substances like lizard excreta, wall gecko tail, mixtures of methylated spirit and Coca-cola drinks, pit latrine or septic tank emissions, gases from urine stored for a few days in air-tight containers, gaseous products from the burning of vehicle tyres, mixtures of flavored carbonated drink and mentholated candy all in a bid to induce hallucinatory effects on themselves [8]. *Agama agama* belong to the family of *Agamidae* is a redheaded rock agama that is also referred to as a common agama or rainbow agama and is mostly found in sub-sahara Africa. *A. agama* size varies from 13 to 30cm (5.1 11.8 inches) in total length. The *A. agama* can be identified by using a white underside, Brown limbs and a tail with a light stripe down the middle. The stripe on the tail typically possesses about six dark patches along its side. Female, adolescents and subordinate males have an olive-green head, while dominant male have a blue body and yellow tail. According to species, agamas live in the forest, in bush, among rocks and on crags, but where their habitat has been cleared or simply invaded by humans. Agamas' hind legs generally are powerful and they can run and leap swiftly when alarmed [9]. *A. agama* is diurnal; they can tolerate higher temperatures than most reptiles but when temperatures approach 38ºC they generally shelter in the shade. *A. agama* are primary insectivorous but have been known to eat small mammals, Reptiles and vegetation. They catch their prey using their tongue, the tip of which is covered by mucous gland that enables the lizard to hold to smaller prey [9]. Lately, the consumption of *A. agama* excreta in Northern Nigerian has become a subject of public concern, largely due to its potential dangers to the health and the overall wellbeing of its users. Modes of abuse/intake of *A. agama* excreta are by inhalation, smoking and drinking a cocktail mixture of it with other substances [10,11]. Revealing the psychoactive and toxic chemical content of *A. agama* excreta is the focus of this study. This is because *A. agama* excreta is now being seriously being abused by young people due to its psychoactive effects. Therefore, determining its chemical constituents will empirically inform decisions on its use. Perhaps, the reason this substance of which very little is known is widely abused may be because it is easily accessible to abusers as it is abundant in the environment; it is easily acquired because it is not expensive; the effects of abusing it have not been explicated as a result of little or no research; and its abuse is not monitored by law enforcement agencies since it is not a regulated substance. Meanwhile, there is serious suspicion that it may have a great toxic potential due to the efficacy with which it induces intoxication on abusers [12].

2.0 MATERIALS AND METHODS

2.1 Materials

The following equipment and reagents were used for this study. All reagents used were of analytical grade except where otherwise stated -Beaker - Pyrex (250ml), Laboratory hand mill - Corona, Mettler - Toledo analytical balance (p/16), Bijou bottle with cover - Emel (500ml), Water thermostatic water bath - Vecstar, Spatula, Conical flask (500ml), Polyethylene film (2 x 2Fq), Glass sample bottles, Chromatographic micro syringe (BS), Gas chromatography/mass spectrograph machine (model QP210SE, Shimadzu, Japan), Sintered glass funnel, Edwards suction pump (1.5hp), Spectrographic grade ethyl alcohol (Sigma Aldrich), Spectrosol (Sigma Aldrich), Methanol (Sigma Aldrich), n- hexane (Sigma Aldrich)

2.2 Methods

2.2.1 Study area

Sample collection was conducted at the Federal University, Otuoke, Ogbia Local Government Area, Bayelsa state, Nigeria, while the Gas Chromatography coupled to Mass Spectrometry (GC-MS) analysis was carried out at the National Agency for Food and Drug Administration and Control (NAFDAC) South-East Zonal Area Laboratory, Agulu, Anambra State. Bayelsa State is located in the Oil-Rich Niger Delta region of Southern Nigeria, with

geographical coordinates of 4°55'N and 6°15'E. The region has a tropical monsoon climate, with temperature fluctuations between 71°F and 87°F, alternating between a wet rainy season and a cloudy dry season [13]. Anambra State is located in the South-East geo-political region of Nigeria. Its geographical coordinates are 6°20'N and 7°00'E. It has a savanna climate with an average yearly temperature of 84.18°F and it is one of the most populated states in Nigeria [14].

2.2.2 Collection of samples

Fresh excreta of *A. agama* were collected from uncompleted buildings, bookshelves, windows and roofs of houses located in the Federal University, Otuoke, Ogbia local government area, Bayelsa state, Nigeria, using hand gloves and placed in small polyethene bags.

2.2.3 Ethical Approval

Ethical clearance was obtained from the animal research ethics committee of the Department of Medical Biochemistry, Faculty of Basic Medical Science, Federal University Otuoke, Bayelsa State.

2.2.4 Preparation of Samples

Collected excreta were spread on a polyethylene film and sun-dried between 9am and 4pm for a period 10days. The dried *A. agama* excreta were pulverized using a laboratory hand mill (Corona) to achieve fine particles with a BS/72 mesh size. The mettler balance was switched on and the beaker was placed on the top of scale pan with the meter reading zero. A 50g aliquot amount of the pulverized sample was weighed and transferred into a bottle and labeled. Then 200ml of ethanol spectrosol was added to the sample. The set up was preserved for 48hrs at room temperature in a dark cupboard. The sample was then filtered with a sintered glass funnel under pressure and the filtrate was received in a 500ml flask. The residue was eluted with additional 50ml of the solvent and the combined filtrate was 225ml. The extract was amber in colour. On a water bath preset at 50°C was placed an aliquot 50ml of the extract contained in a 100ml beaker to dry. Drying was done till the residual volume of the extract was 25ml (volume halved). The concentrated extract was then poured into a 50ml cleaned and dried glass sample bottle with a firm stopper and kept for gas chromatography coupled with mass spectrometry (GC-MS) analysis.

2.2.5 Analysis of Sample

The chemical constituents of the concentrated extracts of A. agama excreta were analyzed using a Gas Chromatography-Mass Spectrometry (GC-MS) system, as outlined by Sparkman et al. [15]. The GC-MS consists of two primary components: the gas chromatograph and the mass spectrometer. The gas chromatograph employs a capillary column, the effectiveness of which depends on its dimensions (length, diameter, and film thickness) and phase properties (e.g., 5% phenyl polysiloxane). Separation of molecules occurs due to differences in their chemical properties and their relative affinities for the stationary phase of the column. As the sample travels through the column, molecules are retained and then elute at different retention times. This separation allows the mass spectrometer to capture, ionize, accelerate, deflect, and detect the ionized molecules individually. The mass spectrometer further fragments each molecule into ionized components, detecting these fragments based on their mass-to-charge ratios. The GC-MS system was prepared by switching on the machine and injecting samples into the sample pot or aspirator pot using a syringe. Subsequently, the inlet tap for the inert carrier gas was opened, followed by activation of the heater to raise the temperature to approximately 230°C in a stable inert atmosphere. The chromatographic process commenced, and the separated fractions were automatically fed into the mass spectrometer for analysis, yielding detailed results and insights. The GC-MS analysis was conducted using a GC Clarus 500 PerkinElmer system equipped with an AOC-20i auto-sampler and interfaced to a mass spectrometer. The system utilized an Elite-1 fused silica capillary column (30 m × 0.25 mm ID × 1 µm film thickness, composed of 100% dimethylpolysiloxane). The instrument operated in electron impact mode at 70 eV, with helium (99.999%) as the carrier gas at a constant flow rate of 1 mL/min. The injection volume was 0.5 µL, with a split ratio of 10:1. The injector temperature was set to 230°C, and the ion source temperature was maintained at 280°C. The oven temperature was programmed as follows: an initial temperature of 110°C was increased at a rate of 10°C/min to 200°C, then at 5°C/min to 280°C, with a final isothermal hold at 280°C. Mass spectra were recorded at 70 eV, with a scan interval of 0.5 seconds and detection of fragments ranging from 40 to 450 Da. The total run time for the GC-MS analysis was 28 minutes.

2.3 Data Analysis

Data generated were analyzed using principal component analysis, cluster analysis and descriptive statistics to determine the percentage of each constituent present.

3.0 RESULTS

The result of the GC-MS analysis revealed 17 compounds which are listed in table 1, along with their nomenclature, chemical formula, structure, molecular weight, retention time and peak area.

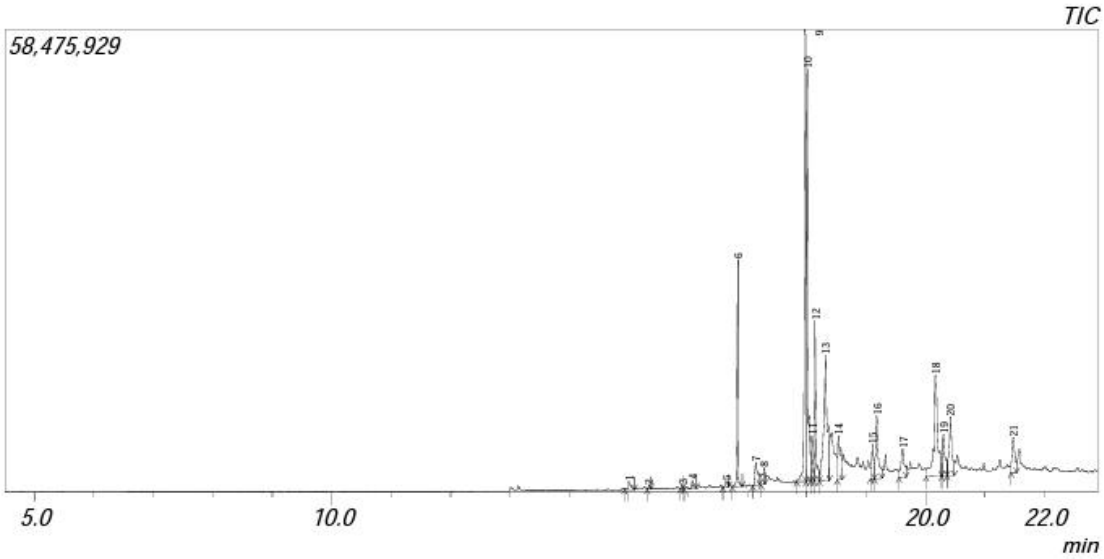
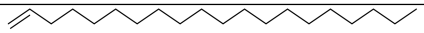
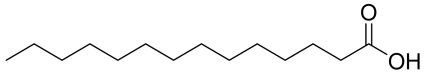
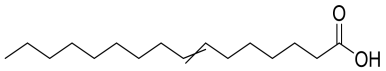
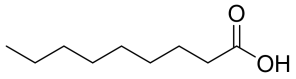
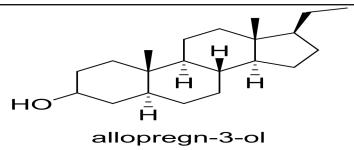
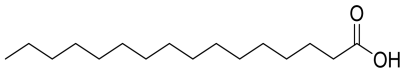
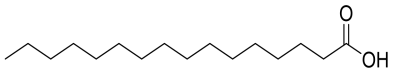
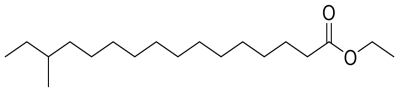
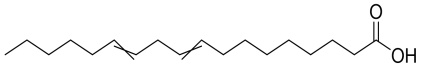
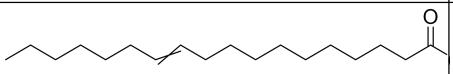
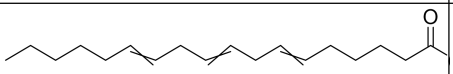
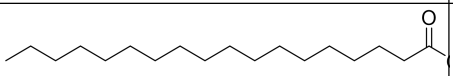
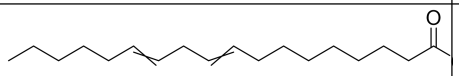
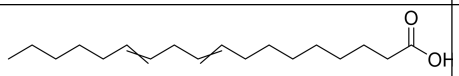
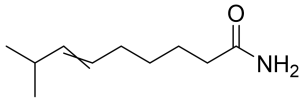
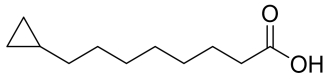
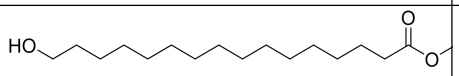


Figure 1: Chromatogram of *A. agama* excreta

Table 1: Chemical constituents of *A. agama* excreta

Hit	Nomenclature & Formula	Structure	Molecular Weight (Da)	Retention Time (Mins)	Peak Area (%)
1	1-Eicosene ($C_{20}H_{40}$)	 1-Eicosene	280	15.008	0.61
2	Tetradecanoic acid ($C_{16}H_{32}O_2$)	 Tetradecanoic acid	256	5.350	0.14
3	7-Hexadecenoic-acid ($C_{17}H_{32}O_2$)	 7-Hexadecenoic-acid	268	15.900	0.08
4	Nonanoic-acid ($C_{16}H_{24}O_2$)	 Nonanoic-acid	248	6.067	0.47
5	Allopregn-3-ol ($C_{27}H_{47}NO$)	 allopregn-3-ol	401	16.642	0.45
6	Hexadecanoic acid ($C_{17}H_{34}O_2$)	 Hexadecanoic acid	270. 44	16.833	6.87

7	n-hexadecanoic acid ($C_{16}H_{32}O_2$)	 n-hexadecanoic acid	256.42	17.133	1.95
8	Ethyl-14-methyl-hexadecanoate ($C_{19}H_{38}O_2$)	 Ethyl-14-methyl-hexadecanoate	298.49	17.283	0.86
9	9,12-Octadecadienoic acid ($C_{19}H_{34}O_2$)	 9,12-Octadecadienoic acid	294	17.967	17.53
10	1-Octadecenoic acid ($C_{19}H_{36}O_2$)	 11-Octadecenoic acid	296	18.008	16.37
11	6,9,12-Octadecatrienoic acid ($C_{19}H_{32}O_2$)	 6,9,12- Octadecatrienoic acid	292	18.083	1.80
12	Methyl stearate ($C_{19}H_{38}O_2$)	 Methyl stearate	298	18.142	5.60
13	9,12- Octadecadienoic acid ($C_{18}H_{32}O_2$)	 9,12- Octadecadienoic acid	254	18.317	12.84
14	15-Hydroxypentadecanoic acid ($C_{15}H_{30}O_3$)	 9,12- Octadecadienoic acid	258	19.175	4.35
15	8-Methyl-6-nonenamide ($C_{10}H_{19}NO$)	 8-Methyl-6-nonenamide	169	19.608	2.71
16	Cyclopropaneoctanoic acid ($C_{22}H_{38}O_2$)	 Cyclopropaneoctanoic acid	334	20.167	11.10
17	Methyl 16-hydroxy-hexadecanoate ($C_{17}H_{34}O_3$)	 Methyl 16-hydroxy-hexadecanoate	286	20.417	4.60

4.0 DISCUSSION

Given the absurdity with which the unconventional use of *A. agama* excreta is perceived and the rate at which it is now being used as an inebriating substance, studies on it has become necessary [16]. From the GC-MS results of *A. agama* excreta, the most abundant constituent by peak area was shown to be 9,12-Octadecadienoic acid (17.53%). The constituent with the highest retention time (20.417mins) is shown to be methyl-16-hydroxyhexadecanoate while the constituent with the highest molecular weight (334 Da) is cyclopropaneoctanoic acid. Identified psychoactive compounds among the discovered constituents of *A. agama* excreta are nonanoic acid, n-hexadecanoic acid and 8-methyl-6-nonenamide. More so, the toxic chemicals identified as constituents of *A. agama* excreta are 1-eicosene, nonanoic acid, n-hexadecanoic acid, 8-methyl-6-nonenamide, 9,12-octadecadienoic acid, hexadecanoic acid, 11-

octadecanoic acid, 15-hydroxypentadecanoic acid and methyl stearate. While a few of the constituents of *A. agama* excreta such as tetradecanoic acid, allopregnan-3-ol and methyl-16-hydroxyhexadecanoic acid and 6,9,12-octadetrienoic acid have not been connected with toxicity most of the constituents are very toxic [17]. Nonanoic acid is an organic compound with nine carbon atoms. It is a colorless oily liquid with an unpleasant rancid odour. It is insoluble in water but very soluble in organic solvents. Studies suggest that nonanoic acid may be more potent than valproic acid as a psychoactive agent, as it induces central nervous system depression. [18,19]. n-Hexadecanoic acid is a psychoactive substance that induces anxiety-like behaviour. It also increases amygdala-base serotonin metabolism. There are reports that 8-methyl-6-nonenamide causes skin irritation, erythema and slight edema in addition to having psychoactive metabolite forms [20]. 1-eicosene has been demonstrated to be harmful to the airways and the liver [21]. Nonanoic acid is also considered a harmful substance due to its ability to cause cough, anemia, liver damage, drowsiness, lassitude and narcosis [19]. 9,12-octadecadienoic acid is a straw-coloured polyunsaturated fatty acid that is highly reactive and rapidly neutralizes bases and reacts with both reducing and oxidizing agents. Exposure to it has been reported to cause irritation of the eyes, the skin and mucous membranes. It also causes nausea and vomiting [22, 23]. Hexadecanoic acid, a 17-carbon fatty acid is associated with the autolysis of membranous structures especially the mitochondria. Studies on rat superior cervical ganglion suggests that hexadecanoic acid induces aortic dilation. Furthermore, it has been found to inhibit phagocytic activity and nitrogen oxide production in cells. It has been also found to cause a reduction in the levels of TNF alpha, PGE2 and IL-10. It has also been linked with the inhibition of NF_kB [23]. 11-octadecanoic acid and 15-hydroxypentadecanoic acid are both connected with the induction of skin, eye and respiratory tract irritation [21]. Methyl stearate has been implicated in the pathology of the gastrointestinal tract in addition to inducing emesis, diarrhea and other adverse health effects similar to that of n-hexadecanoic acid [22]. The findings of this study align with a recent report where a mixture of abused substances was found to impact negatively on kidney function and haematological parameters [24]. The identification of multiple toxic constituents in *A. agama* excreta underscores the potential health risks associated with its increasing use as a psychoactive substance [25]. It has become necessary for relevant government agencies and stakeholders to take deliberate steps to address this fast-growing harmful trend of substance abuse [26].

5.0 CONCLUSION

The toxic compounds identified among the constituents of *A. agama* excreta posit a serious danger to the health of persons that consume it. This finding provides an empirical basis that can serve as a deterrent to the unregulated use *A. agama* excreta by reason of its inebriating effects. Therefore, public awareness campaigns are essential to educate young people on the harmful effects of consuming *A. agama* excreta.

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Authors contributions

Dr. C.G. Ikimi conceptualized the work, did the laboratory analysis and wrote the manuscript. Dr. O.A. Udi conducted the sample collection and processing. All authors read and approved the manuscript.

Conflict of interest

The authors declared no conflict of interests.

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