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Chemical analysis of *Hemidactylus frenatus* (Gekkonidae) Schlegel, 1836 tail: An emerging substance of abuse in Nigeria

[1*]Charles G. Ikimi and [2]Ofovwe O. Ekakitie

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

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ABSTRACT

Background: The campaign against substance abuse requires empirical validation. This study investigates the psychoactive and toxic chemical content of *Hemidactylus frenatus* tail, a substance increasingly abused by Nigerian youth.

Methods: *H. frenatus* tail were collected by cutting off the tails of live *H. frenatus* caught in buildings at 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 *H. frenatus* tail were determined with a Gas Chromatography-Mass Spectrometry (GC-MS) machine.

Results: The result showed twenty-four compounds along with their nomenclature, chemical formula, structure, molecular weight, retention time and peak area, amongst which four are psychoactive and six are toxic. The most abundant constituent by peak area was shown to be 9,12-Octadecadienoic acid (10.56%). The constituent with the highest retention time (21.500mins) is shown to be cyclopentanetridecanoic acid and it was also the constituent with the highest molecular weight (356Da). Identified psychoactive compounds among the discovered constituents in *H. frenatus* tail were 9-octadecenamide, indolizine, 2-furan-methanamine and 2- piperidinone while the toxic chemicals identified among the constituents of *H. frenatus* tail includes indolizine, propanenitrile, 9-octadecenamide, oleic acid, 2-piperidinone and 2-furanmethenamine.

Conclusion: These findings highlight significant health risks and underscore the need for targeted public interventions to curb this trend.

Keywords: GC-MS, *Hemidactylus frenatus*, psychoactive, substance abuse, toxic.

1.0 INTRODUCTION

People abuse psychoactive substances for various reasons which may include curiosity and peer pressure, especially among school children and young adults; recreational purposes; unintended addiction to prescription drugs originally intended for pain relief; spiritual/religious practices or rituals; and as a means of obtaining creative inspiration [1]. Substance abuse disorders are associated with a wide range of short- term and long- term health effects. They can vary depending on the type of drug and route of administration and the individual health at large. Notwithstanding, the effects of drug abuse and dependence can be far- reaching. They have been found to adversely impact almost every organ in the human body with often irreversible deleterious effects [2].

Most commonly abused substances act by temporarily altering a person's neurochemistry, which in turn causes changes in a person's mood, cognition, perception and behaviour [2]. There are many ways in which substance use can affect the brain. Each substance has a specific action on one or more neuro transmitter or neuroreceptor in the brain. Exposure to a psychoactive substance can cause changes in the structure and function of neurons as the nervous system tries to re-establish the homeostasis disrupted by the presence of the substance. Depending on its method of action, a psychoactive substance may block the receptors on the postsynaptic neuron (dendrites), stop

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

block reuptake or affect neurotransmitter synthesis in the presynaptic neuron [3]. There are reports that *H. frenatus* (wall gecko) tails contain intoxication inducing substances and this explains their intake/abuse by some young people [4]. The consumption of *H. frenatus* causes instant intoxication judging from observation of its abusers [5]. The intoxicating effects of the mixture of *H. frenatus* tail is believed to be 50% higher than marijuana and cocaine [6]. The exact cause or substance responsible for the psychoactive effects of this mixture is not yet known. The users are prone to have to some clinical syndromes. The mixture causes addiction as the craving is usually unbearable resulting in nausea, itching of the throat, gums and several body parts and mild trembling. It also causes sedation in the user that lasts at least 10hours [7]. A rare case of the abuse of its mixture by a prisoner with cannabis dependence was contained in an African literature. The report has it that the prisoner shifted to its use during the periods of non-availability of cannabis, and it has since gained popularity as a potent alternative to the regulated cannabis [8]. *H. frenatus* is a gecko that is also known as pacific house gecko, the Asian house gecko, wall gecko, house lizard or moon lizard and is in the family of the Gekkonidae. Most geckos are nocturnal, hiding during the day and foraging for insects at night. They can be seen climbing walls of the house and other buildings in search of insect directed to porchlights and is immediately recognizable by its characteristic chirps. They grow to a length of between 75-150mm (3-6in) and living for about 5 years. These small geckos are non-venomous and not harmful to humans [9]. The body is flattened and the toe pads are divided and the first digit is much smaller than the others, also, the tail has enlarged ventral plates and a denticulate margin. *H. frenatus* are grayish, pinkish, or pale brown with darker flecks. Other characters include divided lamella; dorsum and venter light in coloration, sometimes semi-transparent; a light line through eye; dark lateral stripe may be present. *H. frenatus* has a very distinctive" chuck, chuck, chuck" call which is commonly emitted at dusk and dawn. This call is one of the key indicators that house geckos are present in a particular area. *H. frenatus* exists in Southeast Asia and the Indo-Australia archipelago, the Philippines, Taiwan, and much of Micronesia, Melanesia, and Polynesia. *H. frenatus* are found in Australia, Africa, Mexico, the United States and in several parts of the world. Medium to large geckos may bite if distressed. However, their bite is gentle and will not pierce skin [10]. Modes of abuse/intake of *H. frenatus* tail are by inhalation, smoking and drinking a cocktail mixture of it with other substances [4,6]. Revealing the psychoactive and toxic chemical content of *H. frenatus* tail is the focus of this study. This is because *H. frenatus* tail 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 [11].

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 [12]. 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 [13].

2.2.2 Collection of samples

The tail of H. frenatus were collected by cutting off the tails of life H. frenatus caught in cracks and walls of houses in Otuoke, Ogbia Local Government Area of Bayelsa state, Nigeria, using sterile razor blades and placed in polyethene bags. This mirrors the exact pattern by which the animal is being abused so that the findings of the study

can be directly relevant to its abusers. The Animal Welfare Act of 1985 of the United States of America for research and Institutional Animal Care and Use Committee (IACUC) protocols were stringently adhered to.

2.2.3 Preparation of Samples

The collected H. frenatus tails were spread on a polyethylene film and sun-dried between 9am and 4pm for a period 10days. The dried H. frenatus tail was pulverized with the use of laboratory hand mill (Corona) to obtain a fine particle size of BS/72 mesh. 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 conical flask. The residue was eluted with additional 50ml of the solvent and the combined filtrate was 225ml. The extract was light ash 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.

2.2.4 Analysis of sample

The chemical constituents of the concentrated extracts from the tail of *Hemidactylus frenatus* were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS), as described by Sparkman et al. [14]. The GC-MS consists of a gas chromatograph, which separates molecules based on their chemical properties and affinity for the stationary phase in a capillary column (e.g., 5% phenyl polysiloxane), and a mass spectrometer, which ionizes, fragments, and detects molecules based on their mass-to-charge ratio (m/z). Samples were injected into the GC-MS system using a syringe, with helium (99.999%) as the carrier gas at a flow rate of 1 mL/min. The system was heated to 230°C under an inert atmosphere. The gas chromatograph separated molecules, which eluted at specific retention times, and the separated fractions were transferred automatically to the mass spectrometer for analysis. The GC-MS analysis was performed on a PerkinElmer Clarus 500 system with an Elite-1 fused silica capillary column (30 m × 0.25 mm ID × 1 µm df). The injector temperature was 230°C, the ion source was maintained at 280°C, and the oven was programmed from 110°C (2 min hold) to 200°C at 10°C/min, then to 280°C at 5°C/min, with a final isothermal hold for 9 minutes. Detection was conducted in electron impact mode at 70 eV, with a scan range of 40–450 Da, and a total run time of 28 minutes. The analysis provided detailed information on the chemical composition of the sample, including molecular fragments and their respective mass-to-charge ratios.

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 GC-MS analysis revealed 24 compounds which are listed in table 1, along with their nomenclature, chemical formula, structure, molecular weight, retention time and peak area.

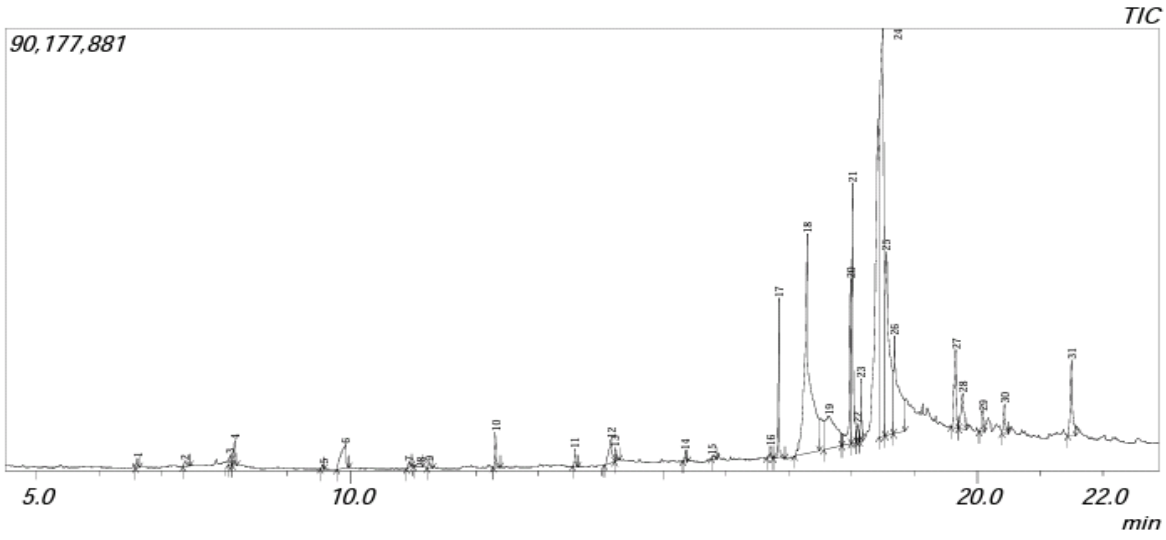
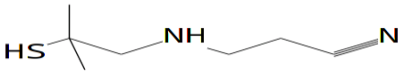
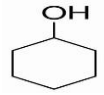
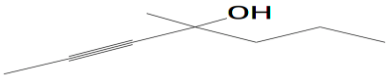
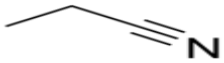
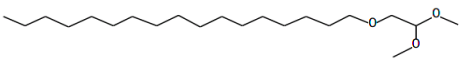
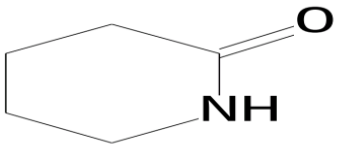
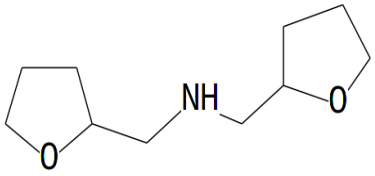
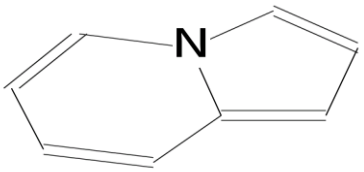
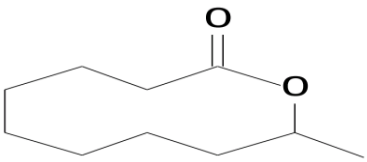
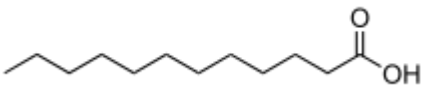
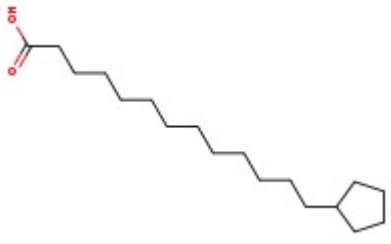
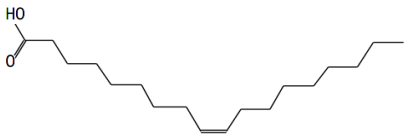
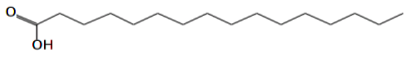
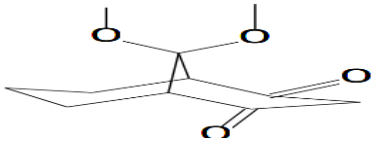


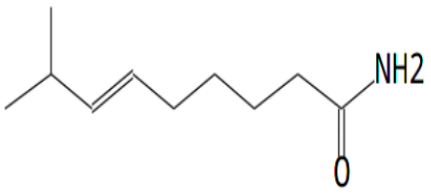
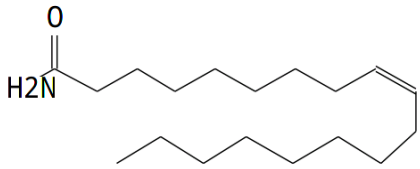
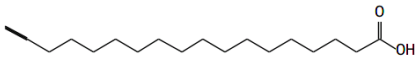
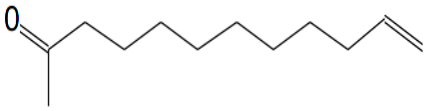
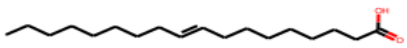
Figure:GC-MS Chromatogram of *H. frenatus* tail

Table 1: Chemical constituents of *H. frenatus* tail

Hit	Nomenclature & Formula	Structure	Molecular Weight	Retention Time	Peak Area (%)
1	N-[2-Cyanoethyl]-2-mercapto-2-methylpropanamide ($C_7H_{14}N_2S$)	 N-[2-Cyanoethyl]-2-mercapto-2-methylpropanamide	158	6.617	0.18
2	Cyclohexanol ($C_9H_{18}O$)	 Cyclohexanol	142	7.375	0.18
3	4-Methyl-2-heptyn-4-ol ($C_8H_{14}O$)	 4-Methyl-2-heptyn-4-ol	126	8.083	0.28
4	Propanenitrile (CH_3CH_2CN)	 Propanenitrile	238	8.158	0.56
5	Chimilether ($C_{21}H_{44}O_3$)	 Chimilether	344	9.550	0.12
6	2-Piperidinone (C_5H_9NO)	 2-Piperidinone	99	9.925	1.99
7	2-Furanmethanamine ($C_{10}H_{19}NO_2$)	 2-Furanmethanamine	185	10.950	0.17
8	2,4(1H,3H)-Pyrimidinedione ($C_4H_6N_2O_2$)		114	11.117	0.23

9	Indolizine (C_8H_7N)	 Indolizine	117	11.250	0.15
10	2-oxecanone ($C_{10}H_{18}O_2$)	 2-oxecanone	170	12.317	0.76
11	Dodecanoic acid ($C_{12}H_{24}O_2$)	 Dodecanoic acid	214	13.583	0.34
12	Hexadecanoic acid ($C_{16}H_{32}O_2$)	 Hexadecanoic acid	284	14.233	2.86
13	13-cyclopentyltridecanoic acid ($C_{18}H_{34}O_2$)	 13-cyclopentyltridecanoic	296	15.342	1.33

14	Oleic acid ($C_{18}H_{34}O_2$)	 Oleic acid	282	15.792	0.14
15	7- hexadecenoic acid ($C_{16}H_{30}O_2$)	 7- hexadecenoic acid	268	16.700	0.29
16	n- hexadecanoic acid ($C_{16}H_{32}O_2$)	 n- hexadecanoic acid	256	17.283	5.82
17	9,12-Octadecadienoic acid ($C_{18}H_{32}O_2$)	 9,12-Octadecadienoic acid	294	17.975	10.56
18	11-octadecenoic acid ($C_{18}H_{34}O_2$)	 11-octadecenoic acid	296	18.008	5.60
19	9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione ($C_{11}H_{16}O_4$)	 9,9-Dimethoxybicyclo [3.3.1] nona-2,4-dione	212	18.092	0.41

20	8-methyl-6-nonenamide ($C_{10}H_{19}NO$)	 8-methyl-6-nonenamide	169	18.683	7.57
21	9-Octadecenamide ($C_{18}H_{35}NO$)	 9-octadecenamide	281	19.650	2.35
22	17- Octadecynoic acid ($C_{18}H_{32}O_2$)	 17- Octadecynoic acid	280	19.758	1.67
23	11-Dodecen-2-one ($C_{12}H_{22}O$)	 11-Dodecen-2-one	182	20.083	0.72
24	9-Octadecenoic acid ($C_{18}H_{34}O_2$)	 9-Octadecenoic acid	356	21.500	2.87

4.0 DISCUSSION

The spate at which H. frenatus tail is being used unconventionally necessitated an investigation of its constituents [15]. From the GC-MS results of H. frenatus tail, the most abundant constituent by peak area was shown to be 9,12-Octadecadienoic acid (10.56%). The constituent with the highest retention time (21.500mins) is shown to be cyclopentanetridecanoic acid and it is also the constituent with the highest molecular weight (356Da). Identified psychoactive compounds among the discovered constituents in H. frenatus tail were 9-octadecenamide, indolizine, 2-furan-methanamine and 2- piperidinone. More so, toxic chemicals identified as constituents of H. frenatus tail includes indolizine, propanenitrile, 9-Octadecenamide, oleic acid, 2-piperidinone and 2-furan methenamine. Indolizines are potent disruptors of protein synthesis. They inhibit enzyme activity and acts as calcium entry blockers in cardiovascular activity Indolizines are also a histamine (H3) receptor antagonist. The derivatives of indolizine were found to exhibit cytotoxic and central nervous system depressant activity [16,17]. Propanenitrile causes sore throat, chest tightness, shortness of breath, unconsciousness, respiratory and cardiac arrest. It induces structural changes in the cells of the duodenum and stomach. It is also associated with bringing about ulcerogenic effect [18]. Increased oleic acid level in the membranes of red blood cell have been associated with increased risk of breast cancer [19]. 9-Octadecenamide has been shown to accumulate in the cerebrospinal fluid and it causes drowsiness [20]. It has also been implicated in the etiology of cannabinoid-regulated depression [21]. 2-piperidinone, a derivative of piperidine, classified as a lactam. It is a known psychotropic agent with cytotoxic potentials [22,23,24]. 2-furan methenamine are and most furan containing chemicals are reported to be very to be very harmful. They are hepatotoxic and carcinogenic [25,26]. The findings of this study make it empirical to prognosticate that the now rampant act of the unconventional use of H. frenatus tail by youths in Nigeria for its inebriating effects shall adversely impact on the health of its abusers as validated by a recent study on the impact of chemical mixtures on immunological indices [27]. This makes it imperative for appropriate legislations and health policies for addressing the current trend in animal abuse to be considered by the concerned government agencies and highly impacted stakeholders like school authorities.

5.0 CONCLUSION

The indiscriminate use H. frenatus tail for its psychoactive properties constitute a threat to the health of the numerous young people who abuse it given its discovered harmful chemical content. If left unchecked, the current trend in substance abuse in Nigeria may lead to an increased incidence of morbidity. This information should be deployed in organized health sensitization schemes to discourage young people from consuming *H. frenatus* tail.

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Authors contributions

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

Ethical Approval

Ethical clearance was obtained from the animal research ethics committee of the Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Federal University Otuoke, Bayelsa State. The Animal Welfare Act of 1985 of the United States of America for research and Institutional Animal Care and Use Committee (IACUC) protocols were stringently adhered to.

Conflict of interest

The authors declared no conflict of interests.

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