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Research Article

Fatal Intoxication of 1-(Thiophen-2-Yl)-2-Methylaminopropane (2-MPA): A Case Report

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Abstract

A 27-year-old man drunk several large pints and had used some novel psychoactive substances (NPS) was found dead in his room with some plastic packets, where two of them were opened and one sealed contained white tablets (jumping beans). The tablets were found adjacent to the dead body and the postmortem indicated sudden cardiac death through cardiac dysrhythmia but there was no medical history of previous heart disease. Accordingly, the cardiac blood sample and the packets of jumping beans were collected and analysis was carried out for drug intoxication. The microscopic and gas chromatography-mass spectrometric (GC-MS) techniques suggested the presence of NPS suspected drug in the decedent's cardiac blood. Moreover, the presence of methiopropamine (MPA) in the suspected seized drug was confirmed using a Fourier Transform Infrared (FTIR) spectrometer. The proton nuclear magnetic resonance (1H-NMR) spectrometric analysis confirmed that the structural isomer 2-MPA was responsible for the cause of the death. The concentration of 2-MPA in the decedent's blood was 752±26 ng/mL. Therefore, the cause of death was intoxication of 2-MPA.

Keywords: Intoxication; 2-MPA; New psychoactive substances

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Introduction

New Psychoactive Substances (NPS) appearing on the drug marked have been a massive threat in the last two decades [1]. Because using NPS drugs in the recreational area has significantly increased throughout the world [2]. However, recently in several countries this drug has been regulated as a narcotics drug, Methiopropamine (MPA) is chemically known as 1-(thiophen-2-yl)-2-methylaminopropan, belongs to the NPS, as can be seen from (Figure 1), it is structurally and pharmacologically an analog to methamphetamine drugs [3-5]. It is commonly known as a street name called "the Jumping Beans". It was investigated for the first time in Finland as a recreational drug [5,6]. It is a Central Nervous System (CNS) stimulant that shows stimulation, attentiveness, and rise of concentration and energy because it functions as a norepinephrine and dopamine reuptake inhibitor and as a serotonin reuptake inhibitor [5,7]. Since 2011, it was synthesized clandestinely and started to appear on the online drug markets in different parts of the world [5,8,9]. Until now the drug is not under control by many countries, which allows drug users to bypass drug laws to get a "legal high" [9]. However, being closely related to methamphetamine, it is likely that MPA is associated with substantial adverse effects even sudden death [10].

There is a lack of available scientific data in the literature regarding the pharmacodynamics and pharmacokinetics of a methiopropamine drug [9,11]. There are only a few cases published in scientific journals related to the acute toxicity of MPA drugs [10,11]. However, another study [12], proposed a possible metabolism of MPA which involves N-demethylation and hydroxylation at the side chain and the thiophene ring followed by glucuronidation and/or sulphation. Yet the therapeutic use of MPA is not recognized by any party [5]. However, the side effects of MPA such as tachycardia, insomnia, and chest pain were reported [11].

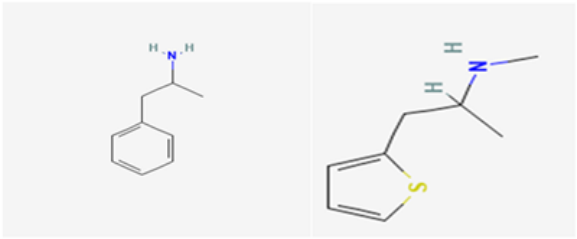

Figure 1: Chemical structure of Amphetamine and Methiopropamine (MPA).

The majority of MPA acute toxicity data are available in the form of a report from different internet discussion forums. However, a study by [11] reported MPA detection in the urine at a concentration of 400 ng/mL. Similarly, [13] MPA in the blood of the deceased was found at a concentration of 9.5 ng/mL. The acute toxicity of MPA in a peripheral blood sample taken 3 days after death was detected at a concentration level of 38 mg/L [10]. According to the report [5], MPA can create a potential risk to drug users and public health in general. There is also a great concern about the possibility of the recreational drug users ingested this drug with alcoholic cocktails of drugs which may lead to drug-drug interactions and making the risk more serious [14]. Therefore, the aim of this study is to identify that 2-MPA was the cause of the death of the 27-year-old man. For this purpose, a human cardiac blood sample from a forensic case and jumping beans were analyzed using the GC-MS, FTIR, and 1H-NMR.

Case report

A 27-years-old man was found vomiting, unconscious in his flat following a night out with friends. An opened packet of jumping beans supposedly drugs were found on the table close to the body. The 27-year-old was brought to a local emergency hospital after his parents found him, unconscious, in his bedroom. At the hospital, he was found to be unresponsive, with a low mental state and lack of coordination. After observation, he presented an elevated pulse rate. Attempts to save the man were unsuccessful. His close friends reported that they had drunk several pints of lager and had used some NPS. This was a relatively normal night out for the group and they considered the drugs to be safe and part of having a 'good night out'. The post-mortem report indicated sudden cardiac death through cardiac dysrhythmia. The decedent's medical history does not show any previous heart disease. Biological specimens were taken and submitted to the toxicology laboratory for analysis.

Post-mortem specimen collection

A sample of heart blood, one sealed and two opened packets containing tablets with commercial name jumping beans were received from the crime scene investigator and submitted for analysis to the Rwanda Forensic Institute (RFI).

Materials and Experimental Methods

Chemicals and reagents

All the reagents used in the assay named 2-MPA standard, amphetamine standard, methanol, ethyl acetate, phosphate buffer, acetonitrile, and ammonium hydroxide were analytical reagent grades obtained from Fisher Scientific (Loughborough, UK). Deionized water (18 MΩcm) was used throughout the analyses.

Instrumentation

The Clarus 600 system gas GC equipped with an auto-sampler and MS detector from Perkin Elmer (Shelton, USA) was used for the identification and quantification of the target analyte. The separation was performed with SLBTM (30m × 0.25mm × 0.25µm) column (Supelco®). The amount of sample injected was 1 µL with split (10:1) mode. The injector temperature was adjusted to 250 °C and the initial oven temperature was 100 °C. The oven temperature was ramped from 100 °C min^{-1} to 300 °C and held for 0.33 min and the total run time was 7 min. The carrier gas was helium at a constant flow rate of 1 mL/min with an initial rate of velocity adjusted 45.5 cms^{-1}. The samples were weighed by using a semi-micro analytical balance (A&D Company Limited (Bradford, UK) with a measurement precision of 0.1 mg.

Physical examination of the drug was performed using the microscopic technique (Carl Zeiss Microimaging GmbH, (Göttingen, Germany). The FTIR spectrometer Specac's Golden Gate™, (Orpington, UK) with a scan range of 400-4000 cm^{-1}, scan number of 16, resolution of 16, scan speed 0.2 cm-1and correction H_2O/CO_2 was used as confirmatory analysis. The 1H-NMR (Bruker Ascend 500, Massachusetts, USA) was used to record the proton NMR of the target compound. Both the 2-MPA standard and powdered jumping bean were dissolved in 600 µL of deuterated methanol.

Toxicological screening

From the postmortem sudden cardiac death through cardiac dysrhythmia, as the decedent didn't have any medical history of previous heart disease a systematic toxicological screening of NPS on cardiac blood and seized drug was performed. As the NPSs are mainly responsible for cardiac dysrhythmia sudden death determination of specific suspected drug of abuse, which could likely be, NPS from post-mortem cardiac blood sample was analyzed by GC-MS after appropriate sample pretreatment procedures.

Solid phase extraction (SPE) of drug from heart blood

A copolymerized sorbent SPE cartridge (CLEAN SCREEN® DAU, Chicago, USA) was inserted into a vacuum manifold and preconditioned with 3 mL of methanol, followed by 3 mL of deionized water and 1 mL of 0.1 M phosphate buffer (pH=6.0). The flow rate of the pump was regulated until a small amount of solvent left over the stationary phase. The prepared 1 mL blood sample was loaded to the SPE columns, and the sample vials were cleaned with deionized water and reloaded to the SPE cartridges. Then, the sample was washed with 2 mL of deionized water and 2 mL of 0.1 M aqueous acetic acid, washed with 3 mL of methanol, and let the pump run for 10 min. Finally, the SPE cartridge was dried under a vacuum and washed with 2 mL of ethyl acetate, acetonitrile, and ammonium hydroxide (78:20:2). The extracted sample was collected in 10 vials and evaporated to dryness under a stream of nitrogen and reconstituted with 50 µL ethyl acetate sonicated for 2 min, transferred to GC vials and injected.

Screening of suspected tablet

Two pieces of solid oral tablets of jumping bean were homogenously powdered using a mortar and pestle. Then the morphological characteristics study of the jumping bean was carried out using microscopy. For GC-MS analysis, about 13 mg of the powdered drug was accurately weighed dissolved in 1mL of methanol, and vortexed for 1 min. Then it was ultra-sonicated and centrifuged (130 × 100 rpm) for 5 min, and filtered through the 0.45 µm filter. About 10 µL of the extract was then transferred into GC vial, diluted to 1 mL with methanol, and analyzed by GC-MS. The insoluble residue part of the suspected drug was evaporated to dryness in the Eppendorf tube and preserved for further analysis. Further screening test was performed on the suspected powdered drug using the microscopic technique to examine the morphological structure.

Preparation of calibration solutions

Five standard calibration solutions with the concentration range (0, 200, 400, 800, and 1000 ng/mL) were prepared in 1 mL blank

blood solution by transferring an appropriate volume of 5 µg/mL of MPA standard solution along with 50 µL of 50 µg/mL amphetamine (IS). Then 2 mL of 0.1 M phosphate buffer (pH=6) was added and vortexed for 30 s.

Positive controls and unknown blood samples

Following the same procedure for standard solutions, three positive control samples with a concentration of 500 ng/mL were prepared following the same procedure without adding the analyte of interest (MPA) in 1 mL GC vial. Duplicate analyte blood samples were also prepared following the same procedure as the positive control samples.

Microscopic identification of the tablet

The powdered jumping bean was taken up on the microscopic slide without using any reagent.

Results and Discussion

The morphological analysis of the suspected drug using microscopic technique observed that some aggregate precipitates were seen which were similar to amphetamine structures that are transparent precipitation with some clusters of plates (Figure 2). Similar microcrystalline structure response has been reported by [15] for amphetamines. This finding suggested that the morphological characteristics of the jumping bean showed a resemblance to amphetamine and helped to carry out the confirmation study.

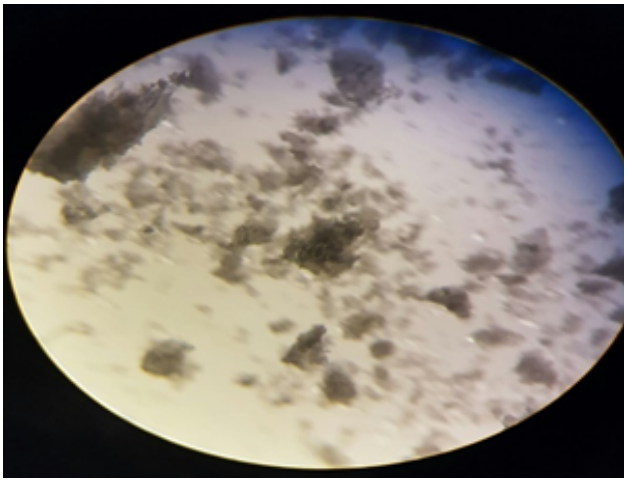

Figure 2: Microcrystalline result of suspected drug tested without using reagent (taken at 10× magnifications).

GC-MS screening of postmortem heart blood sample and suspected drug

The suspected drug was preliminarily analyzed in a postmortem heart blood sample by positive-ion EI screening and compared with the standard MPA. The treated blood samples were injected and one major peak at a retention time (tR) of 2.88 min was obtained (Figure 3A). Besides, a sharp peak at 3.44 min was eluted which could probably be from the column. For the pure standard 2-MPA, the experimental result showed that one major peak at similar tR 2.97 min was eluted. Therefore, the suspected drug detected in the decedent's blood sample could be likely the MPA. However, the standard sample gave two peaks (Figure 4A) co-eluted at a tR of 2.97 and 3.12 min, which could be due to less purity or stability problems.

The extracted suspected drug tablet was analyzed using the same chromatographic conditions and gave three peaks at 2.88, 5.75, and 6.31 min (Figure 3B). The major peak which eluted at 2.88 min was the parent drug peak. However, the other two peaks which eluted at 5.75 and 6.31 min could probably be additives used to make the drugs. Therefore, from the results obtained using GC-MS analyses of decedent blood, suspected drug tablet, and standard MPA, it was possible to deduce the decedent had ingested the suspected drug table as the major peak was eluted at a similar retention time of 2.88 min for the three samples under investigation.

The parent ion for the suspected drug and standard MPA was obtained at (*m/z* 58). The mass spectrum for the suspected drug showed two fragment peaks one large abundant fragment ion at (m/z 58) and one small fragment peak obtained at (m/z=97) with low abundant fragmentation (Figure 3C). A similar result was obtained by [16]. The standard MPA also gave two similar fragment ions at (m/z 58 and m/z 97) for the peaks eluted at tR of 2.97 min and 3.12 min (Figure 4B), where the difference could be due to less purity or stability problems. For screening purposes, the comparison of the mass spectra of the suspected drug from the crime scene (Figure 3C) and standard MPA (Figure 4C) have shown similarities. This finding suggested that the drug found in blood sample screening is similar in chemical composition to the standard MPA.

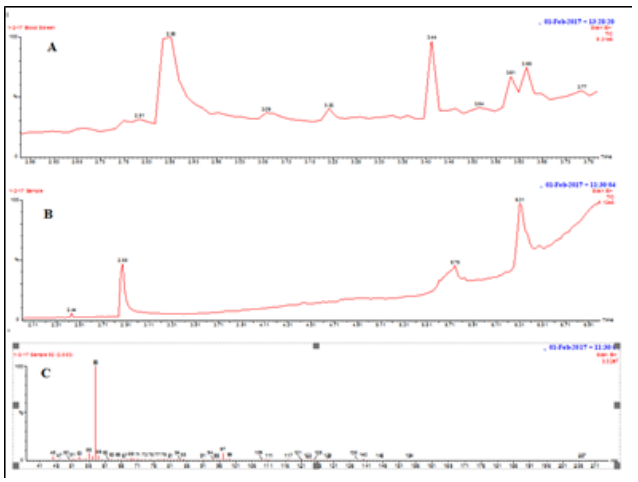

Figure 3: The chromatogram of: blood sample (A), suspected drug from tablet (B), and mass spectra of suspected drug (C).

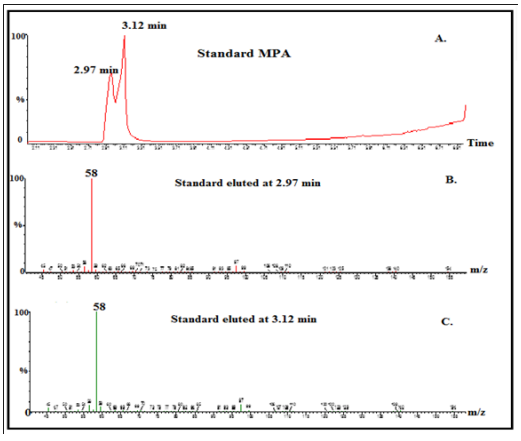

Figure 4: Chromatogram for standard MPA (A), electron ionisation mass spectrum of the standard eluted at 2.97 min (B) and electron ionisation mass spectrum of standard eluted at 3.12 min.

Quantification of the drug blood sample using GC-MS

Following the same chromatographic conditions for standard MPA and suspected jumping bean samples, the estimation of the methiopropamine concentration in the decedent blood was obtained using fortified blind serum samples with MPA at five different concentrations with methamphetamine as IS. Accordingly, the cardiac blood yielded 752 ng/mL of 2-MPA, which was responsible for the intoxication of the decedent.

Method Validations

The adopted method was partially validated to assess if the existing analytical method was suitable for the determination of the MPA drugs. In this study, a quantitative method was validated for linearity, limit of detection (LOD), and precision and accuracy parameters according to ICH guidelines [17]. The correlation coefficient (r^2) of the calibration curve of standard solutions (Figure 5), was 0.996 with regression equation of Y= 0.0082x -0.269. The LOD of the method was determined based on a series of calibration solutions of the MPA drug-fortified from 200-1000 ng/mL. Accordingly, the LOD of the method was obtained to be 98 ng/mL. The precision of the methods was performed by analyzing three control samples at a concentration of 500 ng/mL in triplicate. The% RSD was found to be 2%, showing the system suitability was passed and well accepted as per the ICH guideline. The accuracy (% bias for the three positive control samples spiked as 500 ng/mL was found to be ≤ 1.6%. The accuracy level was ≤ 5% and the percent recoveries determined for all standard samples under investigation ranged from 93% to 103%, which is within the acceptance criteria of the ICH guidelines.

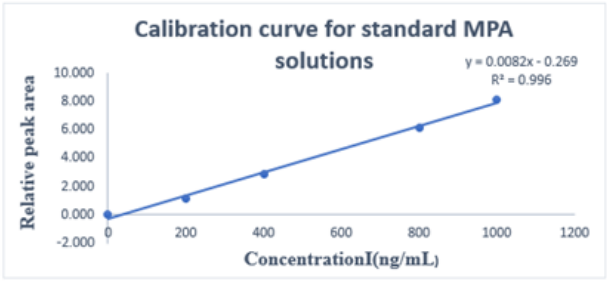

Figure 5: Linear regression for MPA in blood sample and determined GC-MS.

FTIR analysis of standard MPA, powdered jumping bean and insoluble jumping bean residues

The dried residue of an extract of jumping bean powder was taken up for FTIR analysis and the result was compared with the standard MPA and the powdered jumping bean drug. The FTIR spectrum (Figure 6) of standard MPA-HCl has several common absorption peaks with powdered jumping beans, unlike the insoluble jumping bean residue. This result confirmed that the insoluble jumping bean residue does not contain any MPA drugs. However, the standard MPA- HCl and the suspected drug have major peaks are 3000 cm-1, 2964 cm^{-1}, 2717 cm^{-1}, 2453 cm^{-1}, 1384 cm^{-1}, 1049 cm^{-1}, and 704 cm^{-1}. From the FTIR absorption band of the standard MPA and suspected drug, the predominant absorption band provides strong evidence that the suspected drug was MPA. However, the insoluble residue sample does not have an MPA drug, but rather other species which could be cellulose, infant milk, and sucrose.

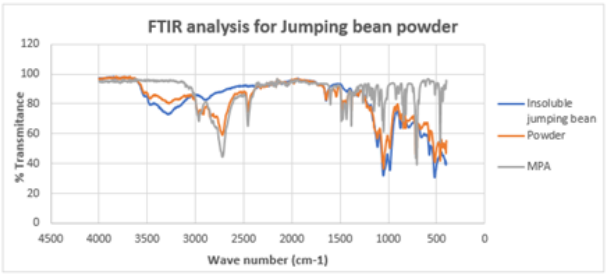

Figure 6: Infrared spectrum of MPA standard, insoluble jumping bean and powdered jumping bean.

From the FTIR spectral search, the algorithms suggested a possible comparison of each spectrum between the jumping bean powder and standard MPA (Table 1). Accordingly, 1–0 as the scale was used, with 1 being the best possible match between the standard and the sample. Thus, the pure standard MPA resulted in 0.996, which indicates the purity of the standard sample. However, the jumping bean powder resulted in 0.542 MPA-HCl, which indicates the jumping bean powder, was not pure and consisted of different additives and cutting agents of street drugs. Besides, with the FTIR analysis, though it was confirmed the presence of MPA in the suspected seized drug, it was difficult to confirm definitively whether the suspected drug was 2-MPA or 3-MPA structural isomers. Thus, further confirmatory analysis was carried out using 1H-NMR to identify if the suspected drug was a 2-MPA or 3-MPA.

FTIR analysis		
MPA standard HCl	**Jumping bean powdered**	**Insoluble residue**
0.996 Methiopropamine HCL	0.563 Cellulose	0.697 cellulose
0.985 Methiopropamine	0.542 Methiopropamine HCL	0.577 Infant Milk
0.469 Hydroxy- Methyl Isopropyl	0.542 Sucrose	0.515 sucrose
0.458Fluorome thamphetamine	0.535 D-Amphetamine SO_4^{2-}	0.434 D-Amphetamine SO_4^{2-}
0.453 Fluorome thamphetamine	0.534 Methiopropamine	0.405 D-Fructose
Standard MPA	MPA detected	No MPA detected

Table 1: FT-IR Search Algorithm–Assessing the Quality of a Match.

NMR analysis of MPA standard and suspected drug

As a further confirmatory analysis, 1H-NMR was also used for analyzing standard MPA and suspected drugs after extracting with deuterated methanol without further purification. Accordingly, with the 1H-NMR it was definitively achieved that the suspect drug was a single substance that was 2-MPA but not a mixture of isomers with 3-MPA (Figures 7&8) (Table 2).

Conclusion

The preliminary presumptive blood test revealed that the suspected drug gave a positive result with GC-MS screening analysis. Besides, the FTIR and NMR revealed that the suspected drug was 2-MPA. The quantitative determination of MPA was performed using

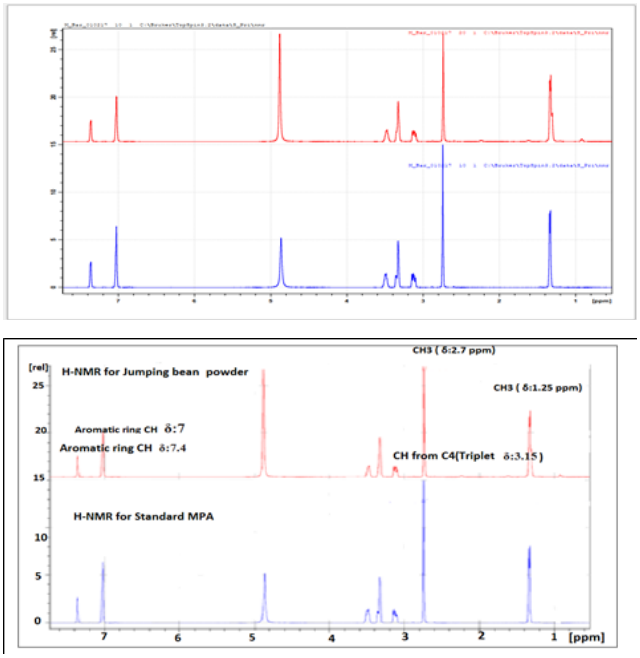

Figure 7: 1H-NMR analyses for standard MPA and jumping bean powder (MPA).

Nodes	shift	Base + Inc.	Comment (ppm rel. to TMS)
CH_3	1.25	0.86	Methyl
CH_3	1	21.13	Methyl
Aromatic CH	7.5	4.59	2-thiophene (Doublet)
Aromatic CH	7.0	9.48	2-thiophene(Triplet)
CH_2	3.5	4.91	Methylene
CH	3.15	5.11	Methine (Doublet)
NH	2.7	4.8	Amine

Table 2: Protocol of the 1H-NMR Predictions.

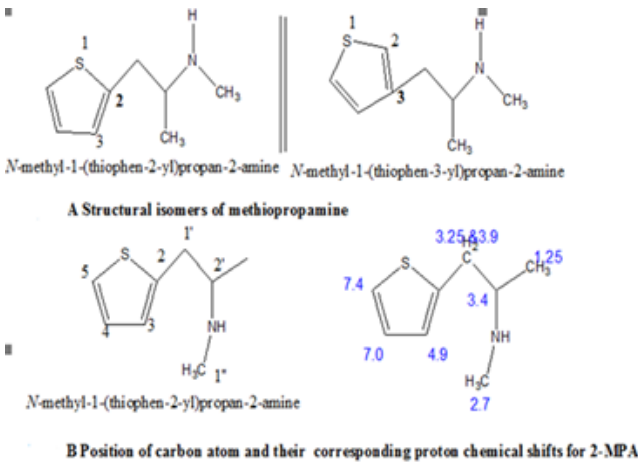

Figure 8: Structural formula and the structural isomers of MPA.

GC-MS and showed that a high concentration of MPA (752 ng/mL), which is greater than 400 ng/mL was reported as acute toxic was obtained in the cardiac blood. Thus, the cause of death was certified as acute 2-MPA intoxication.

Ethical approval

Ethical approval for this study was obtained from the research project committees operating within the Rwanda Forensic Institute (RFI).

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The work received no external funding.

CRediT authorship contribution statement

Libargachew Demlie Mihretu: Investigation, conceptualization, methodology, writing original draft; Asfaw Gebretsadik Gebru: methodology, writing-review and editing; Kebede Nigussie Mekonnen: conceptualization, supervision, methodology, writing-review and editing; Charles Karangwa: conceptualization, supervision, resources, methodology, writing-review and editing; Abraha Gebrekidan Asgedom: methodology, writing-review and editing Justin Kabera: supervision, resources, methodology, writing-review and editing Eliphaz Niyonizera: GC-MS Analysis, methodology, and editing; Tesfamariam Teklu Gebretsadik: FT-IR Interpretation, methodology, writing-review and editing; Amanual Hadera Tesfay: conceptualization, methodology, and editing; Salve Habumugisha: FT-IR Analysis, methodology, and editing Gebremariam Tewelemedhin Gebremariam: blood sample collection, supervision and editing.

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Declaration of Conflict of Interest

The authors declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data available as supplementary information.

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