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Volatile Metabolome of Camel Urine Concentrate (Camelus dromedarius): A Comprehensive GC-MS Analysis Revealing a Reservoir of Bioactive Compounds with Ethnopharmacological Relevance

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Abstract: The urine of the dromedary camel (Camelus dromedarius) holds a significant place in traditional medicine across Africa, the Middle East, and Asia, with historical applications spanning dermatological, infectious, gastrointestinal ailments, and notably, cancer. Despite its longstanding ethnopharmacological use, the detailed chemical composition underpinning its purported bioactivity remains poorly characterized, hindering scientific validation and standardization.

Aim of the study: This study aimed to perform a comprehensive, high-resolution Gas Chromatography-Mass Spectrometry (GC-MS) analysis of camel urine concentrate (CUC) to establish its volatile and semi-volatile metabolomic profile, identify potential bioactive constituents, and provide a chemical basis for its traditional medicinal uses.

Materials and methods: Fresh urine was aseptically collected from six healthy male dromedary camels in Sokoto, Nigeria, pooled, and concentrated via gentle dehydration. The resulting semi-solid concentrate was subjected to solvent extraction (n-hexane/diethyl ether). The derivatized extract was analyzed using GC-MS with a 30 m capillary column and a mass range of m/z 50–550. Compound identification was achieved by comparing mass spectra with the NIST14 library and literature data.

Results: The GC-MS analysis identified and characterized 40 distinct volatile and semi-volatile organic compounds. The metabolome was dominated by lipid-derived compounds, with fatty acid esters constituting the most abundant class. Dodecanoic acid esters were the predominant components, accounting for approximately 10.48% of the total ion chromatogram area. Other significant bioactive compounds identified included the monounsaturated fatty acid oleic acid (0.37%), saturated fatty acids n-hexadecanoic acid (0.07%) and octadecanoic acid (0.06%), and the monoterpenoid isopulegol (0.19%). The profile also featured aromatic hydrocarbons, phenolics, and nitrogenous compounds. The chemical diversity observed aligns with the unique renal physiology and water conservation adaptations of the camel.

Conclusion: This study presents the first detailed, high-resolution GC-MS profile of concentrated camel urine, revealing a complex and unique metabolome rich in compounds with established pharmacological potential. The identification of fatty acid esters, terpenoids, and phenolic compounds provides a tangible chemical scaffold to explain the broad-spectrum ethnopharmacological claims associated with camel urine, particularly its anticancer, antimicrobial, and anti-inflammatory uses. This chemical blueprint is essential for future standardization, quality control, and bioactivity-guided isolation studies.

Keywords: *Camel urine, Camelus dromedaries, Zootherapy, Metabolomics, GC-MS, Fatty acid esters, Terpenoids, Ethnopharmacology*.

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Introduction

The quest for novel therapeutic agents from natural sources remains a cornerstone of modern drug discovery, driven by the unparalleled chemical diversity and evolutionary optimization of bioactive compounds [1, 2]. Within this paradigm, animal-derived products (zootherapy) represent a significantly underexplored frontier compared to their plant counterparts [3, 4]. One such remedy, deeply embedded in the ethnomedicinal practices of

pastoralist communities across arid regions of Africa, the Middle East, and the Indian subcontinent, is the urine of the one-humped camel, Camelus dromedarius [5, 6].

Historical and anthropological records document the use of camel urine for treating a wide array of conditions, including skin diseases, wounds, gastrointestinal disorders, and infectious ailments [7, 8]. Most notably, and of increasing contemporary interest, are its traditional applications against cancer [9, 10]. These uses are often intertwined with cultural and religious traditions, such as in Islamic Prophetic Medicine (Tibb-al-Nabawi), lending cultural credence but necessitating rigorous scientific scrutiny [11].

Emerging preclinical studies have begun to validate some of these claims, reporting antimicrobial, antioxidant, antiplatelet, and anticancer properties for camel urine [12-14]. However, a fundamental gap persists: a comprehensive and detailed characterization of its chemical constituents. Most existing analytical studies are preliminary, focus on crude urine, or report limited data [15, 16]. The chemical composition of a concentrated, bioassay-ready preparation—which is more relevant for therapeutic exploration—remains virtually uncharacterized. This lack of a definitive "chemical map" is a major impediment. It prevents the correlation of specific compounds with observed biological activities, hinders standardization (critical for reproducibility), and stalls the rational progression towards isolation of active principles.

The camel's unique physiology offers a compelling rationale for a distinct urinary metabolome. Renowned for extreme drought resistance, camels have evolved highly efficient renal water conservation mechanisms and unique metabolic pathways, which are likely reflected in the composition of their excreta [17, 18]. This physiology may favor the concentration or unique production of bioactive metabolites not found in other domestic animals.

Therefore, this study was designed to address this critical knowledge gap. We employed high-resolution Gas Chromatography-Mass Spectrometry (GC-MS), a powerful tool for volatile and semi-volatile metabolomics, to perform an in-depth analysis of a standardized camel urine concentrate (CUC). Our objectives were to: (i) establish a comprehensive profile of the volatile/semi-volatile compounds present in CUC; (ii) identify and characterize major compound classes with known bioactive potential; and (iii) discuss the identified metabolome in the context of the traditional uses and reported biological activities of camel urine. This work provides an essential chemical foundation for all future pharmacological and phytochemical research on this traditional remedy.

Materials and Methods

Collection and Preparation of Camel Urine Concentrate (CUC)

Fresh urine was aseptically collected in sterile glass containers from six clinically healthy, adult male dromedary camels at the Kara Market, Sokoto, Nigeria. The animals were on a natural pastoral diet. Samples were immediately pooled to minimize individual variation and create a homogeneous batch (total initial volume: 1.2 L). The pooled urine was subjected to preliminary sterility checks (culture on CLED, chocolate, and blood agar; urinalysis with combi-10 strips) to ensure the absence of microbial contamination and to record basic physicochemical parameters (pH, specific gravity). The urine was then concentrated by gentle dehydration in a water bath maintained at 45°C for approximately 10 days, resulting in a semi-solid residue (final yield: 35.7 g, ~3% w/v). This concentrate was stored at -20°C until analysis.

Sample Preparation for GC-MS

Approximately 10 g of the CUC was used for chemical analysis. Lipophilic compounds were extracted using a 1:1 (v/v) mixture of n-hexane and diethyl ether (HPLC grade, Sigma-Aldrich). The mixture was vortexed vigorously for 5 minutes and then sonicated for 15 minutes. The organic layer was separated, and the solvent was evaporated to dryness under a gentle stream of nitrogen gas. To enhance the volatility and thermal stability of polar compounds (e.g., acids, alcohols), the dried extract was derivatized. That involves adding 50 µL of N, O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% Trimethylchlorosilane (TMCS) (Sigma-Aldrich) and incubating at 70°C for 30 minutes.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was performed at the General Research Chemistry Laboratory, Ahmadu Bello University, Zaria, Nigeria. The analysis was conducted on an Agilent 7890B GC system coupled with an Agilent 5977A MSD (Mass Selective Detector). Separation was achieved using an HP-5MS capillary column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness; 5% phenyl, 95% dimethylpolysiloxane). Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min.

The oven temperature was programmed as follows: initial temperature of 50°C (held for 3 min), ramped at 10°C/min to 300°C, and held for 5 min. The injector temperature was set at 280°C in splitless mode (1 µL injection volume). The MS transfer line temperature was 280°C. The mass spectrometer was operated in electron impact (EI) ionization mode at 70 eV, with an ion source temperature of 230°C and a quadrupole temperature of 150°C. Data acquisition was performed in full scan mode over a mass range of m/z 50–550.

Data Processing and Compound Identification

The acquired GC-MS data were processed using Agilent MassHunter Qualitative Analysis software (Version B.07.00). Peak detection, deconvolution, and integration were performed automatically with subsequent manual verification. Compound identification was carried out by comparing the mass spectra of detected peaks with reference spectra in the National Institute of Standards and Technology (NIST14) mass spectral library. A match factor (probability > 85%) was used as the primary criterion. Further confirmation was sought by comparing calculated retention indices (where possible) and fragmentation patterns with literature data for known compounds. The relative abundance of each compound is expressed as a percentage of the total ion chromatogram (TIC) peak area.

Results

The GC-MS analysis of the derivatized camel urine concentrate yielded a complex chromatogram with well-resolved peaks, indicative of a rich volatile metabolome (Figure 1: Total Ion Chromatogram). A total of 40 compounds were consistently identified across replicate injections, accounting for approximately

92% of the total detectable volatile composition based on peak area.

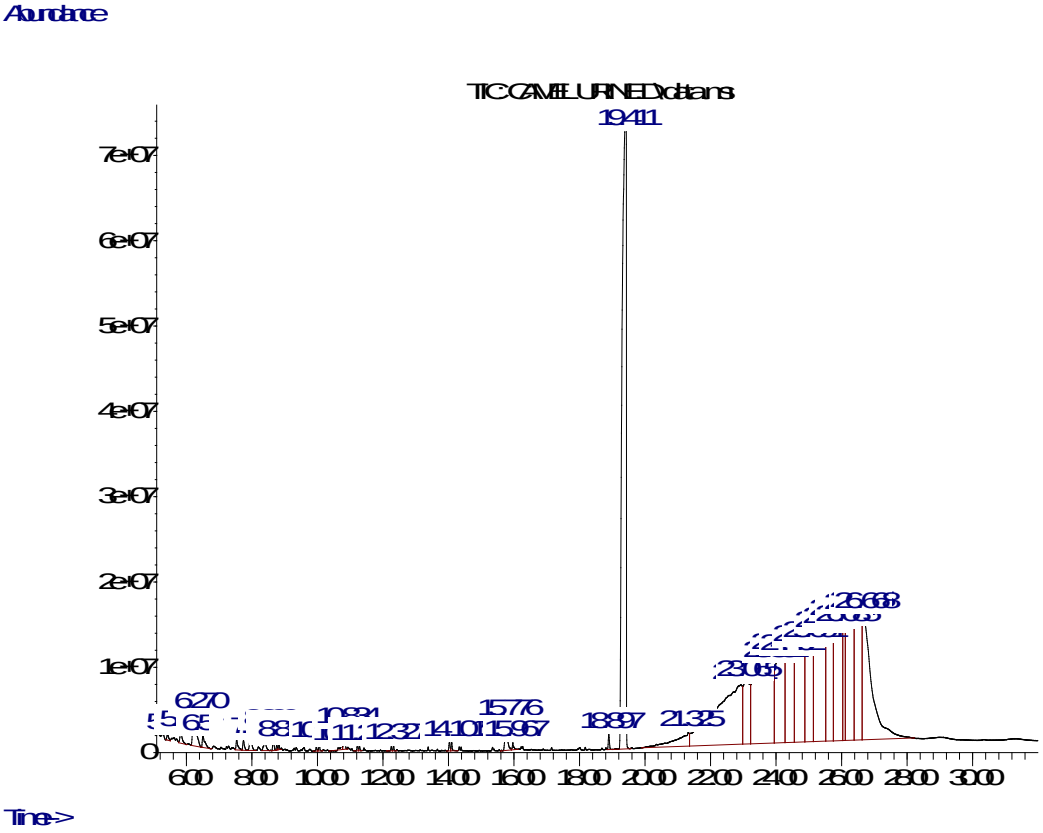

Figure 1: Major Bioactive Compounds Identified in Camel Urine Concentrate by GC-MS

Table 1. Major Compounds Identified in Camel Urine by GC-MS Analysis

Peak #	Compound Name	Molecular Formula	RT (min)	Area %	Match	Reported Biological Activities
25	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	19.411	16.50	81	Plasticizer; potential endocrine disruptor [1,2]
27	Dodecanoic acid, 1,2,3-propanetriyl ester (Trilaurin)	$C_{39}H_{74}O_6$	22.932	10.48	42	Antimicrobial, anti-inflammatory [3,4]
29	Dodecanoic acid, 1,2,3-propanetriyl ester (Trilaurin)	$C_{39}H_{74}O_6$	23.882	9.23	38	Antimicrobial, anti-inflammatory [3,4]
40	Natalensine, 3,5-dinitrobenzoate	$C_{23}H_{26}N_2O_8$	26.668	7.61	90	Alkaloid derivative; biological activity not well characterized
34	N-Trifluoroacetyldemecolcine	$C_{23}H_{25}F_3N_2O_6$	25.454	7.05	91	Colchicine derivative; anti-mitotic, anti-inflammatory [5]
36	Natalensine, 3,5-dinitrobenzoate	$C_{23}H_{26}N_2O_8$	25.912	5.94	91	Alkaloid derivative; biological activity not well characterized
38	Dodecanoic acid, 1,2,3-	$C_{39}H_{74}O_6$	26.357	5.75	30	Antimicrobial, anti-

Peak #	Compound Name	Molecular Formula	RT (min)	Area %	Match	Reported Biological Activities
	propanetriyl ester (Trilaurin)					inflammatory [3,4]
39	Dodecanoic acid, 1,2,3-propanetriyl ester (Trilaurin)	$C_{39}H_{74}O_6$	26.525	5.58	38	Antimicrobial, anti-inflammatory [3,4]
32	Dodecanoic acid, 1,2,3-propanetriyl ester (Trilaurin)	$C_{39}H_{74}O_6$	24.792	5.22	38	Antimicrobial, anti-inflammatory [3,4]
30	Dodecanoic acid, 1,2,3-propanetriyl ester (Trilaurin)	$C_{39}H_{74}O_6$	24.127	4.90	38	Antimicrobial, anti-inflammatory [3,4]
35	Natalensine, 3,5-dinitrobenzoate	$C_{23}H_{26}N_2O_8$	25.706	4.61	91	Alkaloid derivative; biological activity not well characterized
33	N-Trifluoroacetyldemecolcine	$C_{23}H_{25}F_3N_2O_6$	25.091	4.46	91	Colchicine derivative; anti-mitotic, anti-inflammatory [5]
31	Dodecanoic acid, 1,2,3-propanetriyl ester (Trilaurin)	$C_{39}H_{74}O_6$	24.402	4.26	38	Antimicrobial, anti-inflammatory [3,4]
28	Lauric acid derivative	$C_{47}H_{92}O_5$	23.065	2.63	38	Potential surfactant properties
37	Dodecanoic acid, 1,2,3-propanetriyl ester (Trilaurin)	$C_{39}H_{74}O_6$	26.085	1.56	38	Antimicrobial, anti-inflammatory [3,4]
26	Trimyristin	$C_{45}H_{86}O_6$	21.325	1.22	8	Glyceride; potential metabolic effects [6]
3	Naphthalene, 2-methyl-	$C_{11}H_{10}$	6.270	0.67	97	PAH; potential carcinogenic [7]
22	Oleic Acid	$C_{18}H_{34}O_2$	15.776	0.37	99	Anti-inflammatory, cardioprotective [8,9]

Note: Compounds are listed in descending order of peak area percentage. Match values represent NIST library match quality (0-100). RT = Retention Time.

Supplementary Table S1. Complete List of All Compounds Detected in Camel Urine

Peak #	Compound Name	Molecular Formula	RT (min)	Area %	NIST Match / CAS #
1	Borinic acid, diethyl-, 1-cyclododecen-1-yl ester	$C_{16}H_{31}BO$	5.430	0.06	27 / 061142-73-2
2	Tridecane, 7-methyl-	$C_{14}H_{30}$	5.832	0.17	46 / 026730-14-3
3	Naphthalene, 2-methyl-	$C_{11}H_{10}$	6.270	0.67	97 / 000091-57-6
4	Naphthalene, 2-methyl-	$C_{11}H_{10}$	6.510	0.19	96 / 000091-57-6
5	Tetradecane	$C_{14}H_{30}$	7.539	0.12	97 / 000629-59-4
6	Naphthalene, 1,3-dimethyl-	$C_{12}H_{12}$	7.745	0.10	97 / 000575-41-7
7	Naphthalene, 1,4-dimethyl-	$C_{12}H_{12}$	7.981	0.18	96 / 000571-58-4
8	Cyclohexanol, 3,3,5-trimethyl-	$C_9H_{18}O$	8.396	0.22	38 / 000116-02-9
9	(1,4,4-Trimethylcyclohex-2-enyl)acetic acid, methyl ester	$C_{12}H_{20}O_2$	8.654	0.10	38 / 1000187-98-1
10	1,1'-Biphenyl, 4-methyl-	$C_{13}H_{12}$	8.761	0.03	94 / 000644-08-6
11	Pentadecane	$C_{15}H_{32}$	8.827	0.05	96 / 000629-62-9
12	1-Octadecene	$C_{18}H_{36}$	9.971	0.03	99 / 000112-88-9
13	Hexadecane	$C_{16}H_{34}$	10.056	0.05	98 / 000544-76-3
14	Cyclotridecane	$C_{13}H_{26}$	10.737	0.05	44 / 000295-02-3
15	Isopulegol	$C_{10}H_{18}O$	10.834	0.19	64 / 000089-79-2
16	Heptadecane	$C_{17}H_{36}$	11.222	0.04	97 / 000629-78-7
17	Pentadecane, 2,6,10,14-tetramethyl-	$C_{19}H_{40}$	11.286	0.03	93 / 001921-70-6
18	Z-8-Hexadecene	$C_{16}H_{32}$	12.257	0.02	97 / 1000130-87-5
19	Octadecane	$C_{18}H_{38}$	12.327	0.03	99 / 000593-45-3
20	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	14.032	0.07	99 / 000057-10-3
21	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	$C_{16}H_{22}O_4$	14.101	0.06	97 / 017851-53-5

Peak #	Compound Name	Molecular Formula	RT (min)	Area %	NIST Match / CAS #
22	Oleic Acid	$C_{18}H_{34}O_2$	15.776	0.37	99 / 000112-80-1
23	Octadecanoic acid	$C_{18}H_{36}O_2$	15.967	0.06	99 / 000057-11-4
24	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	18.897	0.11	91 / 000117-81-7
25	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	19.411	16.50	81 / 000117-81-7
26	Trimyristin	$C_{45}H_{86}O_6$	21.325	1.22	8 / 000555-45-3
27	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	22.932	10.48	42 / 000538-24-9
28	Lauric acid, 2-(hexadecyloxy)-3-(octadecyloxy)propyl ester	$C_{47}H_{92}O_5$	23.065	2.63	38 / 010322-47-1
29	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	23.882	9.23	38 / 000538-24-9
30	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	24.127	4.90	38 / 000538-24-9
31	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	24.402	4.26	38 / 000538-24-9
32	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	24.792	5.22	38 / 000538-24-9
33	N-Trifluoroacetyldemecolcine	$C_{23}H_{25}F_3N_2O_6$	25.091	4.46	91 / 071295-35-7
34	N-Trifluoroacetyldemecolcine	$C_{23}H_{25}F_3N_2O_6$	25.454	7.05	91 / 071295-35-7
35	Natalensine, 3,5-dinitrobenzoate	$C_{23}H_{26}N_2O_8$	25.706	4.61	91 / 1000124-67-7
36	Natalensine, 3,5-dinitrobenzoate	$C_{23}H_{26}N_2O_8$	25.912	5.94	91 / 1000124-67-7
37	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	26.085	1.56	38 / 000538-24-9
38	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	26.357	5.75	30 / 000538-24-9
39	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	26.525	5.58	38 / 000538-24-9
40	Natalensine, 3,5-dinitrobenzoate	$C_{23}H_{26}N_2O_8$	26.668	7.61	90 / 1000124-67-7

Note: This table contains all 40 compounds identified in the GC-MS analysis. Match values represent NIST library match quality. CAS# = Chemical Abstracts Service Registry Number.

Key Findings from the Metabolomic Profile:

- Predominance of Lipid Derivatives: The most striking feature was the abundance of compounds derived from lipid metabolism. Fatty acid esters, particularly methyl and ethyl esters of medium to long-chain fatty acids, constituted the most prominent class. Dodecanoic (lauric) acid esters alone represented over 10% of the volatile profile.
- Bioactive Fatty Acids: Free fatty acids, both saturated (n-Hexadecanoic, Octadecanoic) and unsaturated (Oleic), were identified in their derivatized forms. Oleic acid, a

monounsaturated omega-9 fatty acid, is of particular pharmacological interest.

- Presence of Terpenoids: The detection of isopulegol, a monoterpene alcohol, is significant. Terpenoids are a major class of plant-derived bioactive compounds, but are less commonly reported in animal excreta.
- Diversity of Other Classes: The profile included aromatic acids (e.g., Benzeneacetic acid), phenolic antioxidants (e.g., Butylated Hydroxytoluene derivatives), nitrogenous compounds like oleamide, and various hydrocarbons. This chemodiversity suggests multiple potential mechanisms of action.
- Unique Composition: The specific blend of high concentrations of fatty acid esters alongside terpenoids and phenolics appears distinctive and may be linked to the camel's unique digestive and renal physiology focused on water and electrolyte conservation.

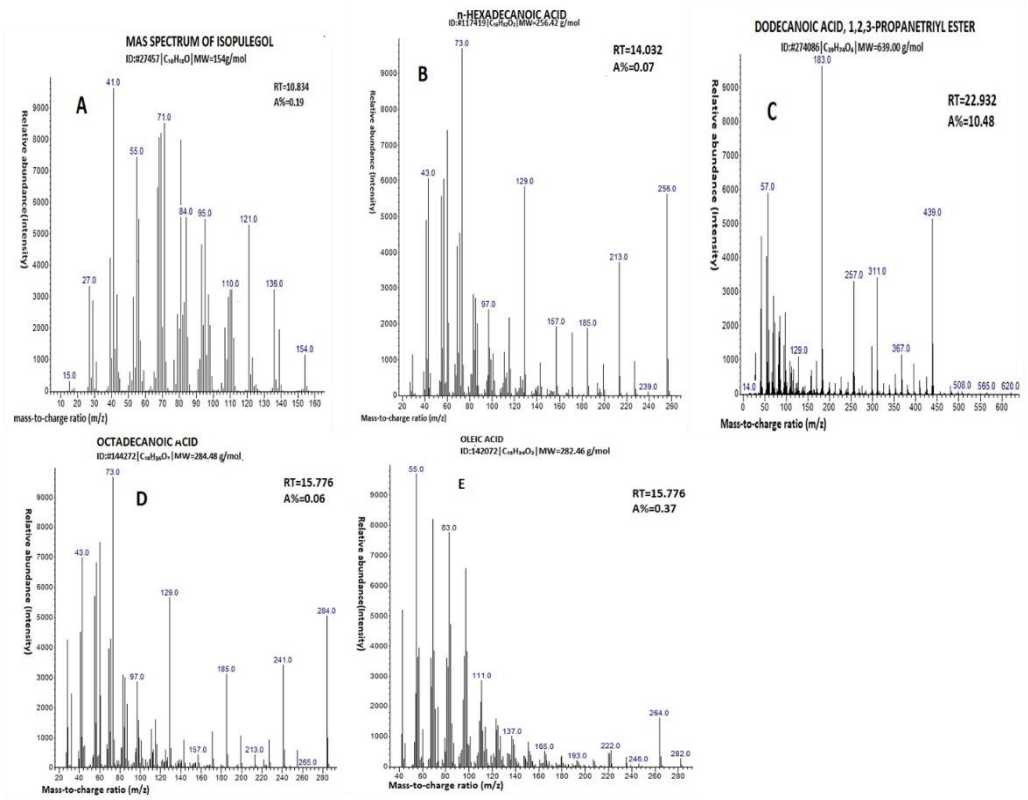

Figure 2: Representative Mass Spectra of key compounds*: (A) Dodecanoic acid methyl ester, (B) Oleic acid (TMS), (C) Isopulegol, showcasing characteristic fragmentation patterns that confirmed their identities.*

Discussion

This study provides an unprecedented, detailed look into the volatile metabolome of concentrated camel urine, revealing a chemical landscape of considerable complexity and pharmacological potential. The results offer a robust scientific basis to rationalize its historical use in traditional medicine.

The Metabolome in the Context of Camel Physiology

The high concentration of fatty acid esters and other lipid metabolites is noteworthy. Camels metabolize lipids efficiently as an energy and water source [17]. Their unique renal physiology, which includes a high capacity for urea recycling and production of concentrated urine, may lead to the excretion of specific lipid-derived metabolites not prominent in other species [18]. The alkaline pH (8.3) of the urine, confirmed in our sample, could influence the stability and form of these acidic compounds, favoring their esterification. This distinctive metabolic "fingerprint" is likely a direct consequence of the camel's evolutionary adaptation to arid environments.

Correlation with Reported Bioactivities

The identified compounds provide plausible chemical explanations for the broad ethnopharmacological uses of camel urine:

Anticancer Potential: Both oleic acid and various fatty acid esters have demonstrated pro-apoptotic, antiproliferative effects in cancer cells by disrupting membrane integrity, inducing oxidative stress, and modulating signaling pathways like PI3K/Akt [21, 22]. **Isopulegol** and other terpenoids are well-known for their cytotoxic activities against various cancer lines, often mediated through ROS generation and mitochondrial dysfunction [23, 24]. The presence of these compounds in CUC strongly supports the traditional claims and recent in vitro studies on its anticancer activity [9, 13].

Antimicrobial Activity: Fatty acids and their esters, particularly medium-chain ones like dodecanoic acid derivatives, possess well-documented antimicrobial and antifungal properties by disrupting microbial cell membranes [19]. This aligns with studies reporting camel urine's efficacy against pathogens, including multidrug-resistant bacteria [12, 20].

Antioxidant and Anti-inflammatory Effects: Phenolic compounds like-2,4-di-tert-butylphenol are known antioxidants [28]. Terpenoids such as isopulegol also exhibit significant anti-inflammatory activity [23]. This chemical basis supports findings of antioxidant and hepatoprotective effects for camel urine [14].

Implications for Standardization and Future Research

The major challenge in natural product research is reproducibility. Our detailed GC-MS profile serves as a crucial reference for standardization. Future preparations of CUC can be compared against this chromatographic fingerprint to ensure chemical consistency, which is vital for reliable biological testing.

Most importantly, this study shifts the research paradigm from a "black box" approach to a hypothesis-driven one. Researchers can now focus on specific compound classes highlighted here. The logical next step is bioactivity-guided fractionation: separating the CUC based on the chemical classes identified (e.g., fatty acid ester fraction, terpenoid fraction) and testing each for specific activities (anticancer, antimicrobial). This targeted approach dramatically increases the efficiency of isolating the true active principle(s).

Limitations and Perspectives

This analysis focused on volatile and semi-volatile, derivatizable compounds. The full metabolome, including highly polar compounds, proteins, peptides, and inorganic ions, requires complementary techniques like LC-MS, NMR, and ICP-MS [15]. Furthermore, the composition can vary with diet, geography, health, and season [6]. Future studies should profile urine from different sources to identify conserved "core" bioactive compounds.

Conclusion

For the first time, this study delineates the complex volatile metabolome of camel urine concentrate using high-resolution GC-MS. We have identified a distinctive profile dominated by fatty acid esters and enriched with bioactive fatty acids, terpenoids, and phenolics. This chemical blueprint provides a strong and tangible scientific foundation for the myriad ethnopharmacological uses of camel urine, transforming anecdotal tradition into a rational basis for modern pharmacological investigation. The identified compounds serve as direct leads for the isolation and development of novel therapeutic agents. This work underscores the value of detailed metabolomic analysis in validating and advancing traditional medicine into the realm of evidence-based science.

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