RESEARCH ARTICLE

Phytochemical profiling and quantification of bioactive constituents in lyophilized khat (*Catha edulis* Forsk) leaf extract from central Uganda

[version 1; peer review: 2 approved with reservations]

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v1 **First published:** 09 Jul 2025, **14**:681
https://doi.org/10.12688/f1000research.163345.1
Latest published: 09 Jul 2025, **14**:681
https://doi.org/10.12688/f1000research.163345.1

Abstract

Background

Phenols, flavonoids, alkaloids, tannins, glycosides and volatile oils are only a few bioactive phytochemicals found in most plants including *Catha edulis* Forsk which is consumed daily in most regions of Uganda.

Objective

This study aimed to analyze and quantify the bioactive constituents in lyophilized leaf extract of *Catha edulis* ('*Kasenge*' variety) from central Uganda.

Methods

Total alkaloids, flavonoids, phenolic and saponins contents were determined using spectrophotometric techniques. The bioactive

Open Peer Review

Approval Status ? ?

	1	2
version 1 09 Jul 2025	? view	? view

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compounds in the *Catha edulis* leaves were identified using high-performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GC-MS).

Result

The total alkaloids, phenolics, flavonoids and saponins contents were 0.0063 mg/g atropine equivalent, 0.099 mg/g gallic acid equivalent, 0.047 mg/g quercetin equivalent and saponins 0.69 mg/g diosgenin equivalent. High-performance liquid chromatography (HPLC) fingerprinting detected thirty-four characteristic phytochemical peaks while gas chromatography-mass spectrometry analysis identified twenty-one bioactive compounds including essential fatty acids and diterpenoids known for their antioxidant, antimicrobial, and anti-inflammatory properties with 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z, Z, Z) constituting 35.01%, hexadecanoic acid, ethyl ester and methyl ester accounting for over 21% of the composition, phytol (10.38%), butyl 9,12-octadecadienoate (7.58%) and 2,4-Di-tert-butylphenol (3.22%).

Conclusion

Although the alkaloid content in the *Kasenge* khat variety was lower than that reported in khat from other regions, the phenolic and flavonoid concentrations were comparable, suggesting the potential antioxidant and anti-inflammatory benefits of this variety. Further research is recommended to explore its pharmacological properties and optimize cultivation practices to enhance the yield of bioactive compound.

Keywords
Atropine, Bioactive compound, Catha edulis, Diosgenin, Gallic acid, Khat, Phytochemical.

This article is included in the Plant Science gateway.

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Author roles: Mbina SA: Conceptualization, Investigation, Methodology, Writing – Original Draft Preparation; **Ajayi CO**: Data Curation, Methodology, Supervision, Writing – Original Draft Preparation; **Dayyabu S**: Supervision, Writing – Original Draft Preparation; **Odoma S**: Investigation, Visualization; **Eze ED**: Visualization, Writing – Original Draft Preparation; **Chebet J**: Investigation, Visualization; **Okon MB**: Software, Validation; **Swase TD**: Software, Validation; **Fasogbon IV**: Data Curation, Methodology, Software; **Aja PM**: Investigation, Supervision, Writing – Original Draft Preparation

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

How to cite this article: Mbina SA, Ajayi CO, Dayyabu S *et al.* **Phytochemical profiling and quantification of bioactive constituents in lyophilized khat (*Catha edulis* Forsk) leaf extract from central Uganda [version 1; peer review: 2 approved with reservations]** F1000Research 2025, **14**:681 https://doi.org/10.12688/f1000research.163345.1

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1. Introduction

Plants contain bioactive substances known as phytochemicals which are produced by secondary plant metabolism in response to environmental changes (Zaynab et al., 2018). Phytochemicals protect plants from eternal invasions by pests and insects as well as pollinator attractants (Zaynab et al., 2018). Environmental conditions, soil type, altitude, agricultural practices, light moisture, temperature, plant portion, harvest season, and plant age constitute factors that affect the generation and quantity of bioactive chemicals (Kirk et al., 2010; Mohamed et al., 2023). The biosynthesis of secondary metabolites shows increased production during times of environmental stress which occur during specific seasons. Secondary metabolite production responds either positively or negatively to environmental stress. The manner in which plants react to environmental stresses depends on both the severity of the stress factor and their individual genetic compositions (Liu et al., 2023). Different biological activities have been attributed to secondary metabolites including phenols, flavonoids, alkaloids and saponins. Phenolic compounds have well-known antioxidant actions that lower oxidative stress and decrease possibility of chronic diseases including cancer and cardiovascular diseases (Daglia, 2012). As a subclass of phenolics, flavonoids have three major therapeutic properties; anti-inflammatory, antiviral and antidiabetic properties (Panche et al., 2016). Similarly, scientists recognize alkaloids for their antimicrobial characteristics along with their analgesic properties whereas, saponins are recognized for immune modulation and cholesterol regulation (Francis et al., 2002).

Catha edulis popularly referred to as 'Khat' is a stimulant and medicinal plant that has garnered a lot of interest. In the Celastraceae family, khat is widely cultivated in the Arabian Peninsula and several East African countries (Kiros, 2020). Khat leaves have historically been chewed by people to achieve stimulating effects from their alkaloidal components, cathinone and cathine (Tembrock et al., 2017). The plant possesses psychoactive properties along with various phytochemicals that contain flavonoids and phenolic compounds combined with tannins and saponins which produce antioxidant, antimicrobial and anti-inflammatory effects (Engidawork, 2017). Although there are legal prohibitions in some countries, the production of khat, also generally known as "mairungi" in Uganda, has become a substantial agricultural activity in most places because of its economic significance (Patel, 2015). The Eastern, Central and Western areas of Uganda are major hubs for khat plant (Beckerleg, 2010). The central region of Uganda characterized by its equatorial climate and fertile soils, provides a unique environment for the growth of *Catha edulis.* Despite its widespread use, the bioactive potential of *Catha edulis* remains underexplored, particularly in terms of the context of its chemical composition and medicinal properties. Research in this area has been largely limited to regions outside Uganda, creating a need to investigate how environmental conditions and agricultural practices influence the phytochemical content of the plant. It has also been documented that the main alkaloid, cathinone present in khat is an unstable principle that can degrade after harvesting of the plant and during the pre-extraction processes (Kelly, 2011; Feng et al., 2017). Hence, khat chewers prefer to immediately consume freshly harvested khat leaves to achieve the maximum psychostimulant effect of cathinone. Consequently, it is essential to use suitable pre-extraction procedures like freeze-drying that will minimize the loss of active volatile chemicals when been used for studies. Therefore, this study estimated the total alkaloid, flavonoid, phenolic and saponin contents of freeze-dried (lyophilized) *Kasenge* khat leaf variety (Figure 1) which is cultivated in the Bulyantete parish, Buikwe district in central Uganda.

Figure 1. Freshly harvested '*Kasenge*' khat variety leaves (Primary data).

2. Methods

2.1 Chemical reagents and instruments

2.1.1 Chemicals and reagents

Quercetin (Sigma-Aldrich, USA, Cas No: 117-39-5), gallic acid (Sigma-Aldrich, USA, Cas No: 5996-86-8), diosgenin (Toronto Research chemical, Canada, Cas No: orb1304570) and atropine (Sigma-Aldrich, USA, Cas No: 51-55-8) standards were used to estimate the total flavonoid, phenolic, saponins and alkaloids contents respectively. Ethanol (C_2H_5OH, 99.7%, EMSURE® ACS, ISO, Reagent, Merck Specialities Private Ltd, India, Cat no: 64-17-5) was used for extraction.

2.1.2 Instrumentation

Spectrophotometric measurements were performed using a Jenway® UV-visible spectrophotometer (Biochrom Ltd, Germany, model: 6715), Labconco FreeZone 4,5 Liters Freeze Dry System by Labconco Corporation, Labconco® (Kansas City USA, model: 7750020) was used for lyophilization. The fingerprinting of the *Catha edulis* leaf extract was performed using the Shimadzu Prominence UFLC High-performance liquid chromatography (HPLC) system manufactured by Shimadzu Corporation, Shimadzu® (Kyoto, Japan). A gas chromatography-mass spectrophotometer manufactured by Shimadzu Corporation, Shimadzu® (Shimadzu, Tokyo, Japan, model: GCMS-QP2020 NX) was used for characterization. All reagents were weighed using a RADWAG MYA.21.4Y. P microbalance produced by RADWAG Balances & Scales, RADWAG® (Radom, Poland).

2.2 Plant collection and identification

Fresh *Catha edulis* leaves were collected from a farm in Mayindo village in Bulyantete parish, Kawolo subcounty, Buikwe district in Central Uganda (0.377471, 32.975878(Lat/Lon) 345° north (UBOS, 2020; Figure 2). The leaf was authenticated by a taxonomist before its voucher specimen was deposited at the Herbarium unit of Makerere University, Uganda where accession number: MHU51321 was allocated.

2.3 Preparation of *Catha edulis* leaf extract

Catha edulis leaf was extracted according to method described by Limenie et al. (2020). Little modifications were made. Briefly, 500g of tender leaves were plucked and washed under running tap water with care. The leaves were then freeze-dried at −20 °C for 48h after which they chopped and crushed using a blender. Ethanol (70% v/v) was used in the extraction process to extract bioactive compounds such as alkaloids, flavonoids and phenolic compounds without significant degradation and thus maintaining stability (Nn, 2015). All the crushed leaf materials were placed into a 1000 mL conical flask and 1000 mL 70% v/v (700 mL ethanol and 300 mL of distilled water (7:3v/v ratio)) was poured into the flask to completely cover the crushed leaf materials and wrapped with aluminum foil. The mixture was then placed on 72g rotary shaker in the dark for 48h at 25°C. After shaking, the mixture was filtered using a grade-I Whatman filter paper. The filtrate was placed in a rotary evaporator to remove the organic solvents at a regulated 36°C temperature, 3g rotation, and 240 Pascals of negative pressure.

2.4 Freeze-drying (lyophilization) of *Catha edulis* extract

The phytochemical content of khat plant materials can be altered by post-harvest operations and drying. Locally, after harvesting, growers wrap the harvested leaves in banana leaves to preserve their freshness and reduce the rapid decomposition of cathinone in the leaves preventing extraction because cathinone as the main alkaloid, undergoes decomposition during transportation (Kelly, 2011; Feng et al., 2017). Consequently, it is essential to use suitable pre-extraction procedures to minimize the loss of active volatile chemicals. Freeze-drying (lyophilization) is a drying technique used to remove water from a sample by sublimation under vacuum (Preethi Samyuktha et al., 2025). This process is suitable for drying unstable or heat-sensitive compounds that may be lost if allowed to dry for a long time (Hazarika and Gosztola, 2020). Hence, lyophilization was used to dry the khat leaf extract used in this study. Lyophilized khat extract was obtained based on the method described by Ligor et al. (2022) using (Labconco FreeZone 4,5 Liters Freeze Dry System Model: 7750020). Briefly, the aqueous extract was placed in Falcon tubes and frozen for 12h at temperatures between 40 °C and 50 °C to prevent the formation of large ice crystals. A two-day freeze-drying method extracted approximately 95% of water from the solution using temperature control and vacuum pressure. After the desorption processing the sample was dried (desorption) to eliminate chemically bound water that remained in the dried substance. The dry (lyophilized) *Catha edulis* extract obtained from the process was kept at freezer at -20 °C until it was used for analysis.

2.5 High performance liquid chromatography (HPLC)

The fingerprinting of the *Catha edulis* leaf extract was performed using a UFLC HPLC system Shimadzu® (Kyoto, Japan). The system contains three main components; an online degasser DGU-20A5R, an ultraviolet (UV) detector and a

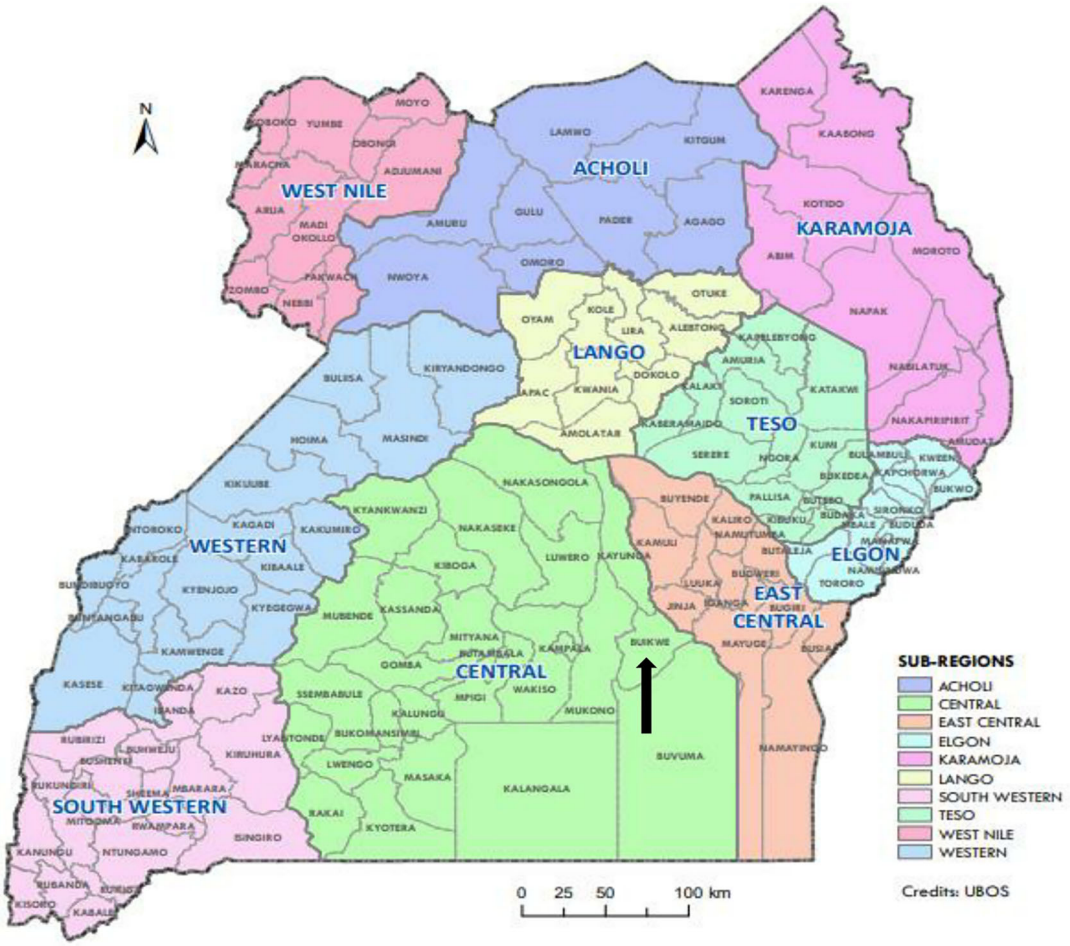

Figure 2. Map of Uganda showing the sub-regions (Uganda National Beureau of Statistics (UBOS), 2020). Black arrow indicates the region where the khat plant (*'Kasenge'*) sample was collected.

Phenomenex Luna C18 column (250 x 4.6 mm, 5 µm) alongside temperature-controlled sample trays and an LC-20AD pump. The reversed-phase HPLC test utilized binary isocratic elution at 1.0 flow rate, 30 °C column temperature and a mobile phase of methanol/acetonitrile/0.01% trifluoroacetic acid (6:1:3). At a detection wavelength of 254nm, the injection volume was 10L. All the solvents used were of HPLC grade.

2.6 Gas chromatography mass spectroscopy (GC-MS) characterization of the *Catha edulis* extract

The presence of various bioactive compounds in the '*Kasenge*' khat variety was identified using GC-MS (Model: GCMS-QP2020 NX, Shimadzu, Tokyo, Japan). The machine used Helium gas (99.999%) as the carrier gas with viscosity compressor time of 0.2 seconds, total flow of 50.0 mL/min at a washing volume 8uL. The analytical conditions for the experiment were optimized as follows: the injection temperature was set at 250.00 °C, while the ion source temperature was maintained at 230.00 °C. The column flow rate was controlled at 1.69 mL/min, with a linear velocity of 47.2 cm/sec. Mass spectrometry was performed with a scan interval of 0.5 seconds, capturing fragments within the mass range of 50-500 m/z (Dalton). The total GC running time was 28.00 minutes and the compounds were identified based on their retention time, retention indices, and mass spectra. To interpret the names, molecular masses and structures of the identified bioactive compounds in khat samples, the spectrum of the unknown compounds was compared with that of the known compounds domiciled in the database of the National Institute of Standard and Technology (NIST) library. The relative percentage concentration of each compound was determined by comparing its average peak area with the total area (Sharma et al., 2015).

2.7 Spectrophotometric determination of compounds in the *Catha edulis* leaf extract

2.7.1 Preparation of standard solutions

Atropine: A standard solution of atropine (1 mg/mL) of was prepared by mixing 10 mg of atropine with 10 mL of methanol. **Quercetin:** A standard solution of quercetin was prepared by mixing 10 mg quercetin with 10 mL methanol. **Gallic acid:** A standard solution of 1mg/mL was prepared by dissolving 10 mg gallic acid in 10 mL of distilled water. **Diosgenin:** A diosgenin standard solution was prepared by mixing 10 mg diosgenin in 10 mL of distilled water.

2.7.2 Determination of total alkaloid content (TAC)

The TAC of *the Catha edulis* extract was determined based on the methods of Shamsa et al. (2008) and Seifried et al. (2007). Briefly, 1 mg/mL of the *Catha edulis* extract was reconstituted with 2N HCl, filtered, and 1 mL of the mixture was added to a separating funnel along with 5 mL of bromocresol green (BCG) solution and phosphate buffer (pH 4.8). The complex formed was consecutively extracted into 1, 2, 3 and 4-mL chloroform with vigorous shaking in 10 mL volumetric flask and made to the volume. Chloroform-containing alkaloids were measured at 415 nm. The total alkaloid content was estimated as atropine (mg/g AE).

2.7.3 Atropine standard curve preparation

Atropine standard solutions (0.01, 0.2, 0.4, 1 and 2 mL) were transferred to five separating funnels that were carefully positioned on the retort stands. To each aliquot in the separating funnel, 4 mL chloroform, 5 mL bromocresol green (BCG) solution and phosphate buffer were added. The complex formed was extracted into 1-, 2-, 3- and 4-mL chloroform in a 10 mL volumetric flask and made to volume. The absorbance of the complex was measured at 415 nm against a blank solution containing all solutions other than atropine. The unknown TAC in the *Catha edulis* extract was estimated using the linear regression curve $y = 0.0027x - 0.0149$; $r^2 = 0.9823$ from atropine following the measurement of absorbance.

2.7.4 Determination of total phenolic content (TPC)

The TPC of the *Catha edulis* extract was estimated using gallic acid as the standard as described by Ainsworth and Gillespie (2007). Aliquots of gallic acid standard solution were accurately measured and placed into 10 mL volumetric flasks at various concentrations (0.01, 0.2, 0.4, 1 and 2 mL). Sodium carbonate (7.5% w/v) and 10% Folin-Ciocalteu reagent were added to 1 ml of 0.5 mg/mL *Catha edulis* extract. Standard concentrations were prepared using similar procedures. All the tubes were incubated for 30 minutes at 40 ^{o}C. The absorbance was measured in triplicates at 760 nm. The line of regression, $y = 0.0136x - 0.0585$; $r^2 = 0.957$ from the gallic acid standard curve was used to determine the TPC.

2.7.5 Determination of total saponins content (TSC)

TSC was determined using the method described by Senguttuvan et al. (2014) with modifications. Each tube contained a normal aliquot (0.01, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mL) of diosgenin and a 0.25 mL aliquot of 1 mg/mL *Catha edulis* extract. Additionally, each tube received 0.25 mL of 8% vanillin reagent and 2.5 mL of 72% sulfuric acid. The mixtures were incubated in a water bath for 15 min at 60 °C and transferred to ice-cold water for 5 min. Absorbance was measured at 560 nm after cooling. The line of regression, $y = 0.0015x + 0.0766$; $r^2 = 0.9746$ from the diosgenin standard curve was used to determine the TSC.

2.7.6 Determination of total flavonoid content (TFC)

TFC of *Catha edulis* was determined using a modified aluminium chloride colorimetric method described by Baba and Malik (2015). A 1 mL aliquot of *Catha edulis* extract (1 mg/mL) was mixed with 3 mL of methanol and vortexed. Subsequently, 0.2 mL of a 10% aluminium chloride ($AlCl_3$) solution and 0.2 mL of 1 M sodium acetate solution were added to the mixture. The solution was incubated for 30 min. Absorbance was measured at 420nm (in triplicate). A calibration curve, $y = 0.006x - 0.0267$; $r^2 = 0.9975$ of standard quercetin at various concentrations (0.01, 0.02, 0.04, 0.1, and 0.2 mg/mL) was used to determine the total amount of flavonoids in the *Catha edulis* extract.

3. Results

3.1 Spectrophotometric determination of phytochemicals in *Catha edulis* extract

Spectrophotometric determination of the total contents of alkaloids, phenols, saponins and flavonoids using the corresponding regression equation of the calibration curves indicated different concentrations in the "*Kasenge*" khat

Table 1. Total alkaloids, phenolic, saponins and flavonoids contents of khat leaf extract.

Phytochemicals	TAC (in AEA)	TPC (in GAE)	TSC (in DES)	TFC (in QEF)
Concentration (mg/g)	0.006 ± 0.0011	0.099 ± 0.0001	0.69 ± 0.0007	0.047 ± 0.0004

Data are expressed as the mean ±SEM and all samples were analyzed in triplicates (n =3). **TAC**: total alkaloid content, **TPC**: total phenolic content, **TSC**: total saponins content, **TFC**: total flavonoid content. **AEA**: atropine equivalent of alkaloids, **GAE**: Gallic acid equivalent of phenolic, **DES**: Diosgenin equivalent of saponins, **QEF**: Quercetin equivalent of flavonoids.

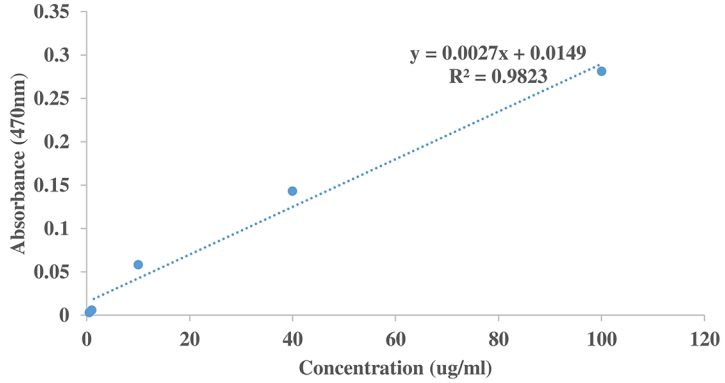

Figure 3. Standard atropine sulphate calibration curve for TAC estimation.

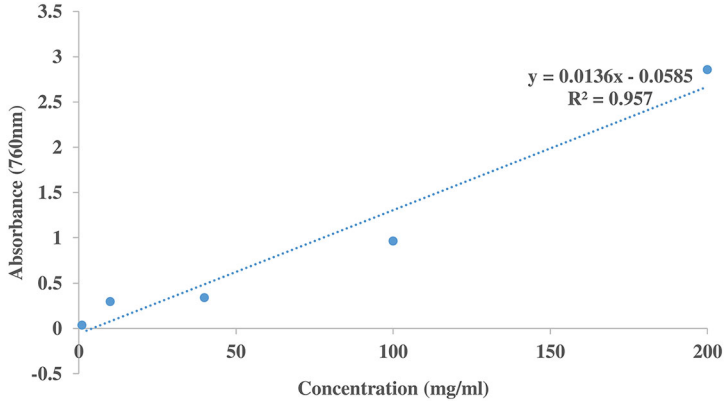

Figure 4. Standard gallic acid calibration curve for TPC estimation.

variety. The total alkaloid content in the khat extract determined from the regression equation of the calibration curve (y = 0.0027x – 0.0149; R^2 = 0.9823) was 0.0063 mg/g AE. The total phenolic content determined using the regression equation of the calibration curve (y = 0.0136x-0.0585; R^2 = 0.957) was 0.099 mg/g GAE. Total saponins content was revealed to be 0.69mg/g DE using the regression equation of calibration curve (y = 0.0015x + 0.0766; R^2 = 0.9746). The total flavonoid content in the sample was 0.047 mg/g, and expressed as quercetin equivalents (QE). This value was calculated using the regression equation (y = 0.006x - 0.0267) derived from the calibration curve, which showed a strong correlation coefficient (R^2 = 0.9975). The results are presented in Table 1 and are illustrated in Figures 3-6.

3.2 High performance liquid chromatography (HPLC) analysis

HPLC analysis of the lyophilized *Catha edulis* extract showed a diverse mixture of phytochemical components in its chromatogram featuring 34 individual peaks (Figure 7). The different phytochemicals in the extract correspond to specific peaks showing their relative abundance based on their retention times measured in minutes. This study used fingerprinting as a chemical characterization method essential for quality control, standardization and comparative analysis between different *Catha edulis* varieties. The examined compounds exhibited retention times ranging from 3.797 to 40.558 min demonstrating the presence of polar and non-polar phytochemicals. Multiple high-intensity peaks

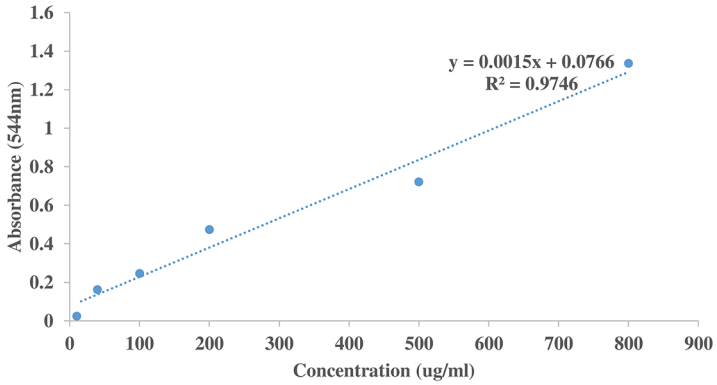

Figure 5. **Standard diosgenin calibration curve for TSC estimation.**

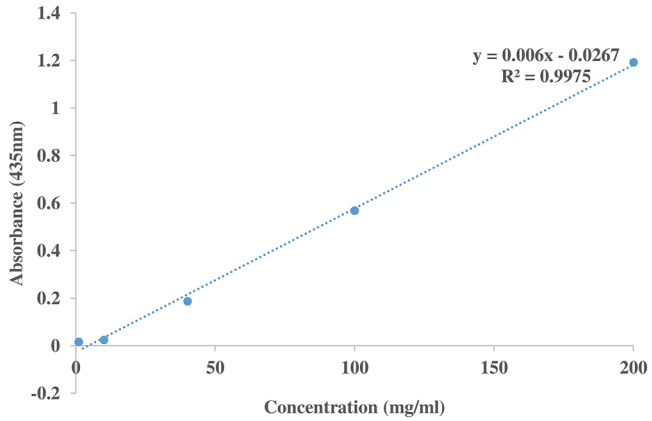

Figure 6. **Standard quercetin calibration curve for TFC estimation.**

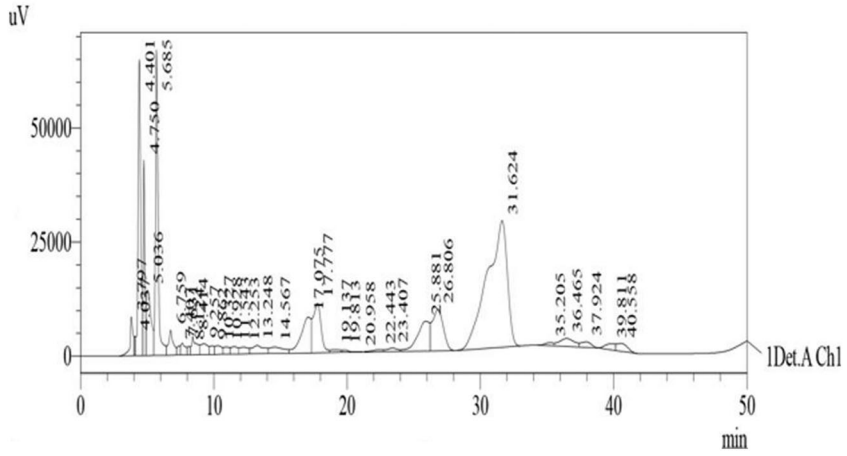

Figure 7. **HPLC chromatogram of chemical constituents in lyophilized *Catha edulis* extract.**

emerged during the analysis at retention times of 4.401, 5.685, 17.777, 26.806 and 31.624 min. This peak at 31.624 minutes demonstrated the highest area percentage (33.048%) indicating its dominant presence in the extract. The peak table showing the retention time of the identified phytochemicals in the khat extract is presented in Table 2 (Mbina et al., 2025).

Table 2. HPLC Peak table showing the retention time of the identified phytochemicals in the khat extract.

Peak#	Retention time	Area	Height	Area %	Height %
1	3.797	131349	8585	1.490	2.834
2	4.037	23154	4259	0.263	1.406
3	4.401	920529	64888	10.442	21.421
4	4.750	327374	42846	3.714	14.145
5	5.036	362899	14344	4.117	4.735
6	5.685	868263	66949	9.850	22.102
7	6.759	135160	5459	1.533	1.802
8	7.407	32006	2026	0.363	0.669
9	7.631	63283	2604	0.718	0.860
10	8.124	24864	1849	0.282	0.610
11	8.414	106962	3991	1.213	1.317
12	9.257	90757	2356	1.030	0.778
13	9.862	44897	1854	0.509	0.612
14	10.327	66049	1889	0.749	0.624
15	10.928	49388	1609	0.560	0.531
16	11.543	59940	1636	0.680	0.540
17	12.253	67997	1524	0.771	0.503
18	13.248	117536	1790	1.333	0.591
19	14.567	106358	1428	1.207	0.471
20	17.075	401001	7821	4.549	2.582
21	17.777	454788	10790	5.159	3.562
22	19.137	30746	618	0.349	0.204
23	19.813	16501	494	0.187	0.163
24	20.958	2801	77	0.032	0.025
25	22.443	25484	463	0.289	0.153
26	23.407	42108	759	0.478	0.251
27	25.881	410182	6519	4.653	2.152
28	26.806	520466	9134	5.904	3.015
29	31.624	2913270	27705	33.048	9.146
30	35.205	30083	663	0.341	0.219
31	36.465	139074	1765	1.578	0.583
32	37.924	64706	1205	0.734	0.398
33	39.811	67957	1329	0.771	0.439
34	40.558	97316	1684	1.104	0.556
Total		8815246	302912	100.000	100.000

3.3 Gas chromatography-mass spectrophotometry (GC-MS) analysis

GC-MS analysis of the lyophilized khat extract revealed a diverse profile of the bioactive compounds. The GC-MS analysis identified 21 peaks with corresponding area percentages. Among the major compounds identified in the *Kasenge* khat variety was 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z, Z, Z), a linolenic acid glycerol ester constituting 35.01% of the total peak area, hexadecanoic acid, ethyl ester and methyl ester, derivatives of palmitic acid collectively accounted for over 21% of the composition, phytol, a diterpene alcohol, constituting 10.38% area, butyl 9,12-octadecadienoate (7.58%) and 2,4-Di-tert-butylphenol with an area percentage of 3.22% (Figure 8 and Table 3).

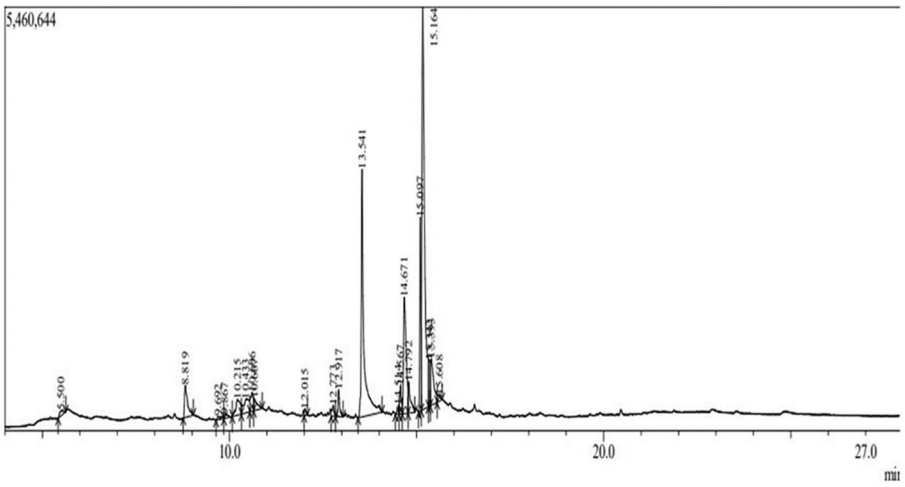

Figure 8. **GC-MS Chromatogram of Lyophilized *Catha edulis* leaf extract.**

Table 3. **Details of phytochemical compounds identified by GC-MS analysis of khat extract.**

Peak	R time	Area	Area %	Name of identified compounds
1	5.500	421104	0.58	alpha. -d-Xylopyranoside, 2,4-O-(ethylboranediyl)
2	8.819	2354837	3.22	2,4-Di-tert-butylphenol
3	9.692	290374	0.40	2-(2-(2-Butoxyethoxy) ethoxy) ethyl 3-methylbut-2-enoate
4	9.867	255130	0.35	Eicosane
5	10.215	1680841	2.30	Methyl 6-O-[1-methylpropyl]-. beta. -d-galactopyranoside
6	10.433	1900695	2.60	Trimethylsilyl 3-methyl-4-[(trimethylsilyl)oxy] benzoate
7	10.606	1229086	1.68	Eicosane
8	10.667	868739	1.19	Tetradecane, 4-ethyl-
9	12.015	224404	0.31	Neophytadiene
10	12.773	513011	0.70	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene
11	12.917	1057451	1.44	Hexadecanoic acid, methyl ester
12	13.541	14970692	20.46	Hexadecanoic acid, ethyl ester
13	14.514	334990	0.46	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z, Z)-
14	14.567	1025351	1.40	6-Octadecenoic acid, methyl ester, (Z)-
15	14.671	7593298	10.38	Phytol
16	14.792	1674166	2.29	Methyl stearate
17	15.097	5545626	7.58	Butyl 9,12-octadecadienoate
18	15.164	25621271	35.01	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z,Z,Z)
19	15.342	1763661	2.41	Dichloroacetic acid, tridec-2-ynyl ester
20	15.393	3436935	4.70	Ethyl 13-methyl-tetradecanoate
21	15.608	421283	0.58	(Z)6-Pentadecen-1-ol

4. Discussion

The current study analyzed the phytochemical profile and quantification of bioactive constituents in the lyophilized khat (*Catha edulis*) leaf extract particularly, the '*Kasenge'* variety from Central Uganda. Spectrophotometric evaluation of the khat leaf extract showed varying amounts of alkaloids, phenolic compounds, flavonoids, and saponins. The analysis revealed the total alkaloid content (TAC) as 0.063mg/g. Generally, documented evidence shows that khat contains alkaloids responsible for its pharmacological and psychostimulant properties (Engidawork, 2017). The concentration of

khat in this research fell below levels found in previous studies of 0.01 to 0.5 mg/g khat alkaloid values recorded in Ethiopia and Yemen (Al-radaa and Abood, 2017). The low alkaloid content of '*Kasenge*' khat could be attributed to environmental factor such as, altitude. The environment in Uganda is characterized by a varied range of altitudes spanning from the lowlands of the Albertine Rift at approximately 600 m above sea level to highland areas of the Rwenzori Mountains of over 5000 m (Akankwasah et al., 2022). This altitudinal change had a significant effect on plants phytochemicals including alkaloids. High-altitude conditions are linked to increased UV radiation and lower temperatures hence, plants located at higher altitude accumulate more alkaloids because environmental stress triggers secondary metabolite synthesis (Liu et al., 2023). Conversely, low-altitude plants may exhibit lower alkaloid levels because of reduced environmental stress. In particular, the central region of Uganda is mainly known to have a low or moderate altitude (1,000 and 1,300 meters above sea level) compared to the higher altitudes found in the western region of the country (Akankwasah et al., 2022; UBOS, 2022). This altitudinal condition in the central region of Uganda may explain its low alkaloid content. The concentration of the main alkaloid, cathinone which is responsible for the stimulant effect of khat has been reported to be influenced by genetic variations, soil fertility, agricultural practices, and drying processes (Kelly, 2011; Feng et al., 2017). The total phenolic content (TPC) and flavonoids content (TFC) were 0.099 and 0.047 mg/g, respectively. The reported values match earlier research discoveries that show khat is rich in phenolic and flavonoids compounds whose antioxidant and anti-inflammatory properties are well-documented (Alsanosy et al., 2020). The TPC and TFC contents in this study were lower levels than those in Yemen khat (IB and TZ varieties) and Ethiopia khat (DMR and HAR varieties) which displayed elevated TPC (IB=0.278mg, TZ= 0.275mg, DMR= 0.333mg and HAR=0.338mg respectively). The Dhamar and Ethiopia varieties on the other hand contained the greatest TFC concentrations at levels of DMR= 88.27mg and HAR= 79.59mg whereas the Yemeni samples had lower TFC levels at IB= 69.50mg and TZ= 70.18mg (Abdelwahab et al., 2015). These TPC and TFC variations can be attributed to earlier environmental conditions, agricultural practices and harvest season (Kirk et al., 2010; Mohamed et al., 2023). The total saponin content (TSC) was highest among the quantified phytochemicals at 0.69 mg/g DES. This result corroborates with the findings by Francis et al. (2002), who reported significant saponin levels in khat, attributing their presence to its potential medicinal benefits such as regulation of cholesterol and modulation the immunity.

In this study, thirty-four (34) characteristic signals were detected by HPLC analysis of khat leaf extract indicating the presence of different chemical compounds in the sample (Figure 7 and Table 2). Multiple peaks in the chromatographic profile show how the secondary metabolites in the extract consist of alkaloids, flavonoids, phenolic compounds, saponins and other phytochemicals. The bioactivity properties of this plant stem from different metabolites that exhibit stimulant behavior alongside antimicrobial and antioxidant effects (Engidawork, 2017; Patel, 2015). The differences in peak intensity together with retention time indicates potential variations in chemical substances. Additionally, GC-MS analysis identified twenty-one (21) bioactive compounds in the khat extract including, 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z, Z, Z), hexadecanoic acid, ethyl ester and methyl ester, phytol, butyl 9,12-octadecadienoate and 2,4-Di-tert-butylphenol constituting major percentage areas (Figure 8, Table 3). 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z, Z, Z), is a linolenic acid glycerol ester reported for its anti-inflammatory and neuroprotective properties (Abdullah et al., 2020) hexadecanoic acid, ethyl ester, methyl ester, derivatives of palmitic acid collectively contribute to antimicrobial activity of khat (Daglia, 2012). Phytol is a diterpene alcohol with known anti-inflammatory properties (Fatima et al., 2017), butyl 9,12-octadecadienoate and 2,4-Di-tert-butylphenol with an area percentage. Similar to previous studies, the GC-MS analysis in this study showed a variation in the number of bioactive constituents identified. For instance, sixteen (16) specific chemical compounds have been identified in khat leaf extract from Yemen (Abdullah et al., 2020), twenty-two (22) identified by (Fatima et al., 2017), twenty-six (26) each and twenty (20) bioactive compounds identified in three different varieties of ethanolic extracts of young leaves of *Catha edulis* from Saudi Arabia (Alsanosy et al., 2020), thirty-nine (39) compounds in *Catha edulis* variety from Djibouti (Fatouma et al., 2023). These variations in GC-MS identified bioactive compounds from the respective regions underscores the fact that types of analytical instrumentation, choice of extraction processes, harvest period, soil and climate could have an influence (Do et al., 2014; Mohamed et al., 2023).

The Spectrophotometric and HPLC analyses in this study detected alkaloids but the GC-MS analysis found trace amounts of alkaloids' derivatives. The sample analysis and injection procedure using GC-MS generates high temperatures that can cause cathinone and cathine to degrade before detection (Kelly, 2011). However, HPLC operates at lower temperatures to maintain the compound stability (Tembrock et al., 2017). The spectrophotometric method detects lower concentrations better than GC-MS because it exhibits higher sensitivity however, GC-MS has a minimum detection threshold (Seifried et al., 2007). The concentration of alkaloids in the *Kasenge* khat extract may have been too low for GC-MS detection but measurable via spectrophotometry. Environmental conditions along with post-harvest processes have been identified in this study to impact phytochemical yields. Moreover, the use of freeze-drying in this study helped preserve most bioactive compounds however, the low concentrations of some volatile compounds may have been due to the handling of the sample during the extraction and analytical processes (Kelly, 2011).

5. Conclusion

This study provides valuable insights into the phytochemical composition of the lyophilized *Catha edulis* leaf extract of the *Kasenge* variety from Central Uganda. The medicinal potential of this extract is more likely because they contain alkaloids at different concentrations along with phenolic compounds, flavonoids and saponins. Multiple compounds with established pharmacological properties were detected using a combination of HPLC and GC-MS. These findings underscore the need for further research on the therapeutic potential of khat, particularly in the development of standardized extracts for medicinal use. Future studies should explore the photochemistry of varieties of khat from different regions of the country to understand the pharmacokinetics and bioavailability of various compounds, their mechanisms of action and potential health benefits.

Ethics and consent

Ethics and consent were not required.

Data availability

Underlying data

Open science framework: Phytochemical profiling and quantification of bioactive constituents in lyophilized khat (*Catha edulis* Forsk) leaf extract from central Uganda. DOI: https://doi.org/10.17605/OSF.IO/6SMFX (Mbina et al., 2025).

The project contains the following underlying data:

- HPLC Peak table showing the retention time of the identified phytochemicals in Khat extract
- Khat_ total alkaloids, phenols, flavonoids & saponins contents.

Acknowledgements

We thank Ms. Rehema Chirindo, Clinical Officer at Kawolo General Hospital, Kawolo, Buikwe district, central Uganda for her commitment and assistance in locating the local khat grower to collect the plant samples for this study from the source.

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Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 06 July 2026

https://doi.org/10.5256/f1000research.179676.r412082

?

Afroz Alam
Banasthali University, Jaipur, Rajasthan, India

Decision: Major Revision
The manuscript has potential value as a phytochemical characterization study of a regional *Catha edulis* variety. However, important methodological details, analytical validation, statistical treatment, and scientific discussion must be strengthened. Addressing the concerns outlined below would substantially improve the rigor, reproducibility, and scientific impact of the study:
Major concerns:

1. **Novelty:** Clearly state the scientific novelty and significance of studying the Kasenge variety compared to previously reported *Catha edulis* varieties.
2. **Plant Material:** Include detailed information on the collection site (GPS coordinates), collection season, altitude, voucher specimen number, plant age, and storage conditions.
3. **Extraction Procedure:** Report the extraction yield, lyophilization conditions, and extraction efficiency.
4. **HPLC Methodology:** Provide complete chromatographic conditions, including column specifications, mobile phase, gradient program, detection wavelength, flow rate, injection volume, and identification of the detected peaks.
5. **GC-MS Validation:** Describe the spectral library used (e.g., NIST), match quality, retention indices, and criteria for compound identification.
6. **Method Validation:** Include calibration curves, linearity, limits of detection (LOD), limits of quantification (LOQ), accuracy, and precision for the quantitative assays.
7. **Statistical Analysis:** Specify the number of biological and analytical replicates, statistical methods, software used, and significance level.
8. **Comparison with Previous Studies:** Expand the discussion by comparing the phytochemical composition with reports from other geographical regions and explaining the observed differences.
9. **Biological Activity:** Since no bioactivity assays were performed, conclusions regarding antioxidant, antimicrobial, and anti-inflammatory properties should be presented cautiously and supported by appropriate literature.
10. **Language and Presentation:** The manuscript requires careful English editing to improve grammar, consistency, and scientific clarity. Minor issues such as changing "Result" to

"Results" and ensuring consistent units should also be corrected.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Phytochemistry, Ethnobotany, Taxonomy

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 23 September 2025

https://doi.org/10.5256/f1000research.179676.r411808

?

Jack Chun-Ting Liu Liu
Stanford University, Stanford, CA, USA

The article examines the phytochemical profile of the leaves of medicinally valuable plant, Catha edulis, using HPLC and GCMS. They quantify total alkaloids, flavonoids, phenolic and saponins contents and found them to be different from those reported in khat from other regions, attributing to environmental factors.

Details for the method section should be clarified:
2.4

- "-40°C and -50°C"?
- “After the desorption processing the sample was dried (desorption)” What does the “desorption” process mean here?
2.5
- injection volume "10 µL"?
- isocratic elution at 1.0 "mL/min"?
2.6
- m/z has no unit "Dalton"
- column temperature during the run not specified

Additional comments:
While the authors identified 34 peaks and 21 peaks using HPLC and GCMS, respectively, additional verification of the identities of these compound would make the work more impactful. For example, using chemical standards to compared against the HPLC traces. Authors should also list the confidence score for the compounds identified by GCMS NIST library. These should be referred to "putatively identified compounds" unless standards are used for identification.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: natural product chemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.