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GC-MS Analysis of Bioactive Compounds in Ethyl Acetate and Chloroform Extracts of *Aloe rabaiensis* Rendle

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A B S T R A C T

Objective: Plants have been a good source of lead compounds for nutraceutical and therapeutic potentials against ailments of human and animal importance since the introduction of man on earth. The lead compounds continue to be precursors in developing drugs with insignificant side effects and replace synthetic medicines. Several phytochemical compounds have been reported in different species of Aloe belonging to the family Asphodelaceae. The established phytoconstituents from the genus Aloe possess several activities, including antiviral, antibacterial, anticancer, neuroprotective, and antioxidant properties. However, there is limited information related to phytochemicals from *Aloe rabaiensis* Rendle.

Methods: This is the first study investigating the phytoconstituents in *A. rabaiensis* from Tanzania. Gas chromatography-mass spectrometry (GC-MS) analysis of *A. rabaiensis* leaf extracts was performed using GC-MS equipment (Agilent Technologies) to identify the phytochemical constituents.

Results: The GC-MS analysis demonstrated the presence of fourteen and six compounds from the leaf's ethyl acetate and chloroform extracts of *A. rabaiensis*, respectively. The compounds belong to different classes, including amines, esters, carboxylic acid, ketone, phenol, alkane and alcohol. No common compound(s) was/were identified from both extracts.

Conclusion: The identified phytoconstituents, backed with reported ethnomedical and ethnoveterinary use of *A. rabaiensis*, are evidence, thus, significant precursors for drug development.

Keywords: *A. rabaiensis*, GC-MS, Phytoconstituents, Pharmacological properties, Plant extract

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Introduction

Plant-based medicines are known for their significant roles in preventing and treating chronic and infectious diseases of humans and animals worldwide [1]. Most people meet their primary healthcare needs in developing countries by depending on medicinal plants [2]. Historically, plants have been a good source of lead compounds essential for nutritional and medical use [3]. There are more than 10,000 plant species in Tanzania; only a few have been investigated for their bioactive compounds and pharmacological activities. About 44 Aloe species have been reported to be found in Tanzania. However, the majority are undocumented for their bioactive compounds [4]. The limited health services and unavailability of affordable medicine in most rural settings in developing countries make investigating active compounds from plants significant for further biological and pharmacological studies.

Aloe rabaiensis Rendle (family *Asphodelaceae*) is a succulent evergreen shrub that grows up to 2 Meters tall. The leaves can grow 30-45 cm long and 3-8cm wider. Geographically, the plant is distributed in Tanzania, Kenya, and Somalia [5]. The ethnomedical survey of the Digo people of Tanzania showed that the exudate of *A. rabaiensis* treats diarrhoea-associated diseases of all kinds. The Giriama people in Kenya use the exudate of *A. rabaiensis* and Acokanthera schimperi as a substance that

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negates the effect of poisons or toxins on a person stabbed with a poisoned arrow [6]. When taken orally, the water extract of *A. rabaiensis* is used to treat an enlarged spleen; however, excess use may cause vomiting and diarrhoea [5]. The laxative property of *A. rabaiensis* is speculated to be similar to Aloe vera due to anthraquinones found in the sap [7]. Despite ethnomedical information related to *A. rabaiensis*, there is limited published literature describing the bioactive compounds in the leaf of *A. rabaiensis*.

Regarding the usefulness and efficacies of active ingredients from different parts of plants against human and animal pathogenic microbes, the study uses the GC-MS machine to analyse the phytoconstituents from *A. rabaiensis* leaf. The identified bioactives will be categorised into classes of phytocompounds for further pharmacological studies.

Materials and Method

Plant sample

The researcher collected leaves of *A. rabaiensis* from around Lake Jipe's northern part of Tanzania (3.34882 S, 37.44202 E at altitude 718 M) from November to December 2020. A Botanist from Tropical Pesticide Research Institute (TPRI), Tanzania, identified and authenticated the plant, and voucher specimen number ARH 403 was deposited in the TPRI herbarium.

Plant sample preparation

Leaves of *A. rabaiensis* were cleaned by running tap water followed by distilled water to remove dust and soil. The leaves were chopped into fine particles using a sharp, sterile knife and dried on the sterilised wire mesh for two weeks in a closed room during the hottest period (34 °C). After 24 h, the leaves on the wire mesh were changed upside down using sterile hands with gloves to avoid contamination by mould growth. The dried leaves were pulverised into fine particles by a milling machine (Swinging Traditional Chinese Machine Pulverizer Diaxiang electronic equipment -DXF- 20D, China). However, the *A. rabaiensis* leaf collected during the rainy season should preferably be dried under the oven to prevent mould growth.

Plant sample extraction

The pulverised *A. rabaiensis* leaf (250 g) was sequentially soaked in chloroform and ethyl acetate (1000 mL each) and placed on the shaker (Dragon lab, USA.) for 48 h. After 48 h, the extracts were filtered using cotton wool and Whatman's No. 1 filter paper. The extracts were concentrated using a rotary evaporator (Heidolph, Germany). The chloroform extract was left in a fumed chamber for 24 h for the solvent to evaporate completely. The concentrated ethyl acetate extract was kept in a water bath (40 °C for 48 h) for the remaining solvent to evaporate. The obtained dried extracts were weighed and stored in the refrigerator at 4 °C until use.

Sample preparation for GC-MS:

1 mg of crude extract of *A. rabaiensis* leaf from ethyl acetate and chloroform was weighed separately and dissolved in 1 mL of respective HPLC grade solvent each. The mixture was vortexed for 3 minutes and centrifuged for 5 min at 10000 r.p.m. A sample was then filtered, and 0.5 mL was loaded in the vials of the autosampler of the GC machine, with every extract preceded by a blank of its respective HPLC grade solvent.

GC-MS Analysis

The GC-MS analysis of secondary metabolites of *A. rabaiensis* leaf extracts used Agilent Technologies GC systems with GC-7890A/ MS-5975C model (Agilent Technologies, USA) connected to HP-5MS with a capillary column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). The electron ionisation system with an ionisation energy of 70eV was involved in the GC-MS machine. Helium gas (99.99%) was used as carrier gas at a constant flow of 1.2 mL/min and injection volume of 1µL of 1% diluted extract in the respective HPLC grade solvent. The initial oven temperature was set at 50 °C to 150 °C with an increased rate of 3 °C/min and a holding time of 10 min. The final temperature was raised to 280 °C at 10 °C/min, and the total running time of the machine was 35 min. The GC-MS machine's interpretation of mass spectrum used the National Institute Standard and Technology (NIST) database with more than 62,000 patterns. The mass spectra of detected compounds from A. rabaiensis leaf extracts were compared with spectra in the NIST library. The comparison provided the chemical name, molecular weight, molecular formula, and structural formula of each detected compound in A. rabaiensis leaf extracts.

Results

GC-MS analysis was used to identify phytochemical compounds present in *A. rabaiensis* leaf. The results of biologically active compounds in ethyl acetate and chloroform extracts of *A. rabaiensis* leaf are presented in Tables 1 and 2. The Identification and characterisation of the bioactive compounds based on their elution order in a HP-5MS column. The elution time, molecular formula, molecular weight, class of the compound, and pharmacological activity are also shown in Tables 1 and 2. The chemical structure of phytochemicals in the above tables are shown in Fig. 1a and 1b, respectively.

Table 1 presents the bioactive compounds in the ethyl acetate extract of *A. rabaiensis*. Esters exhibited higher proportionality among the classes of secondary metabolites observed in ethyl acetate extracts. The identified ester compounds were 2-methylbutyl pentanoate, pentan-3-yl 3-oxobutanoate, pentyl 3-methylbutanoate, 3-methylbutyl 3-methylbutanoate, 2-

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methylbutyl dodecanoate and 3-methylbutyl cyclohexanecarboxylate. Amines, alcohols, alkaloids, alkanes, alkenes, and carboxylic acids were other compounds identified.

Table 2 presents the secondary metabolites in the chloroform extract of *A. rabaiensis* leaf. Of the six classes of compounds identified by GC-MS, alkaloids were two, namely 1,5-dimethyl-2-phenyl-4-propan-2-ylpyrazol-3-one and 2-ethylacridine; others were phenol, ketone, and alkane.

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Table 1. Compounds identified in the ethyl acetate extract of A. rabaiensis leaf in GC-MS

RT	Name of a compound	Molecular weight	Molecular formula	Compound nature	Pharmacological activity	Reference
8.849	O-decyl hydroxylamine	173.3	$C_{10}H_{23}O$	Amine	Anti-inflammatory, antimicrobial	[8,9]
9.175	(2~{R},4~{R})-pentane-1,2,3,4,5-pentol	152.146	$C_5H_{12}O_5$	Alcohol	Antimicrobial	[10]
9.610	2-Methylpyrrolidine	85.15	$C_5H_{11}N$	Alkaloid	anti-inflammatory, anticoagulant	[11]
9.742	2-methylbutyl pentanoate	172.268	$C_{10}H_{20}O_2$	Ester	Antifungal, antibacterial	[12]
10.068	pentan-3-yl 3-oxobutanoate	172.224	$C_9H_{16}O_3$	Ester	Antiviral, antifungal, anticancer, antimicrobial	[13]
10.640	pentyl 3-methylbutanoate	172.268	$C_{10}H_{20}O_2$	Ester	Antioxidant, antimicrobial	[14, 15]
10.640	3-methylbutyl 3-methylbutanoate	172.268	$C_{10}H_{20}O^2$	Ester	Antioxidant, antibacterial	[16,17]
12.632	2-methylbutyl dodecanoate	270.457	$C_{17}H_{34}O_2$	Ester	Antibacterial	[18]
12.969	2,3,4-trimethylhexane	128.259	C_9H_{20}	Alkane	Antibacterial, antifungal, antioxidant	[19, 20]
15.670	3,4-diethylhexane	142.286	$C_{11}H_{22}$	Alkane	Antiviral, antiandrogenic	[21]
17.398	3-methylbutyl cyclohexanecarboxylate	198.306	$C_{12}H_{22}O_2$	Easter	Antibacterial, antifungal	[22, 23]
19.149	3-methyl-4-oxo pentanoic acid	130.143	$C_6H_{10}O_3$	Carboxylic acid	Anticancer, antimicrobial, antioxidant	[24]

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20.425	3,7,11,15-tetramethylhexadec-2-en-1-ol	296.539	$C_{20}H_{40}O$	Alcohol	Antinociceptive, antioxidant	[25]
22.931	5-methyl-2-phenylindolizine	207.276	C15H13N	Alkaloid	Antimicrobial, antioxidant	[26]

Table 2. Compounds identified in the chloroform extract of A. rabaiensis leaf in GC-MS

RT	Name of a compound	Molecular weight	Molecular formula	Compound nature	Pharmacological activity	Reference
9.427	1-cyclohexylbutan-1-one	154.253	$C_{10}H_{18}O$	Ketone	Antimicrobial	[27]
10.068	3,3-dimethyl hexane	114.232	C_8H_{18}	Alkane	Antimicrobial, antioxidant	[28, 29]
11.081	1,1-dichloro-2,2-difluoroethane	134.935	$C_2H_2Cl_2F_2$	Alkane	Antiviral, antibacterial	[30]
13.473	2,4-di-tert -butyl-phenol	206.329	$C_{14}H_{22}O$	Phenol	Antioxidant, antifungal, antibacterial, anticancer	[31- 33]
21.386	1,5-dimethyl-2-phenyl-4-propan-2-ylpyrazol-3-one	230.311	$C_{14}H_{18}N_2O$	Ketone	Anti-inflammatory,	[34]
23.882	2-ethyl acridine	207.276	$C_{15}H_{13}N$	Alkaloid	Antimicrobial, antioxidant	[35]

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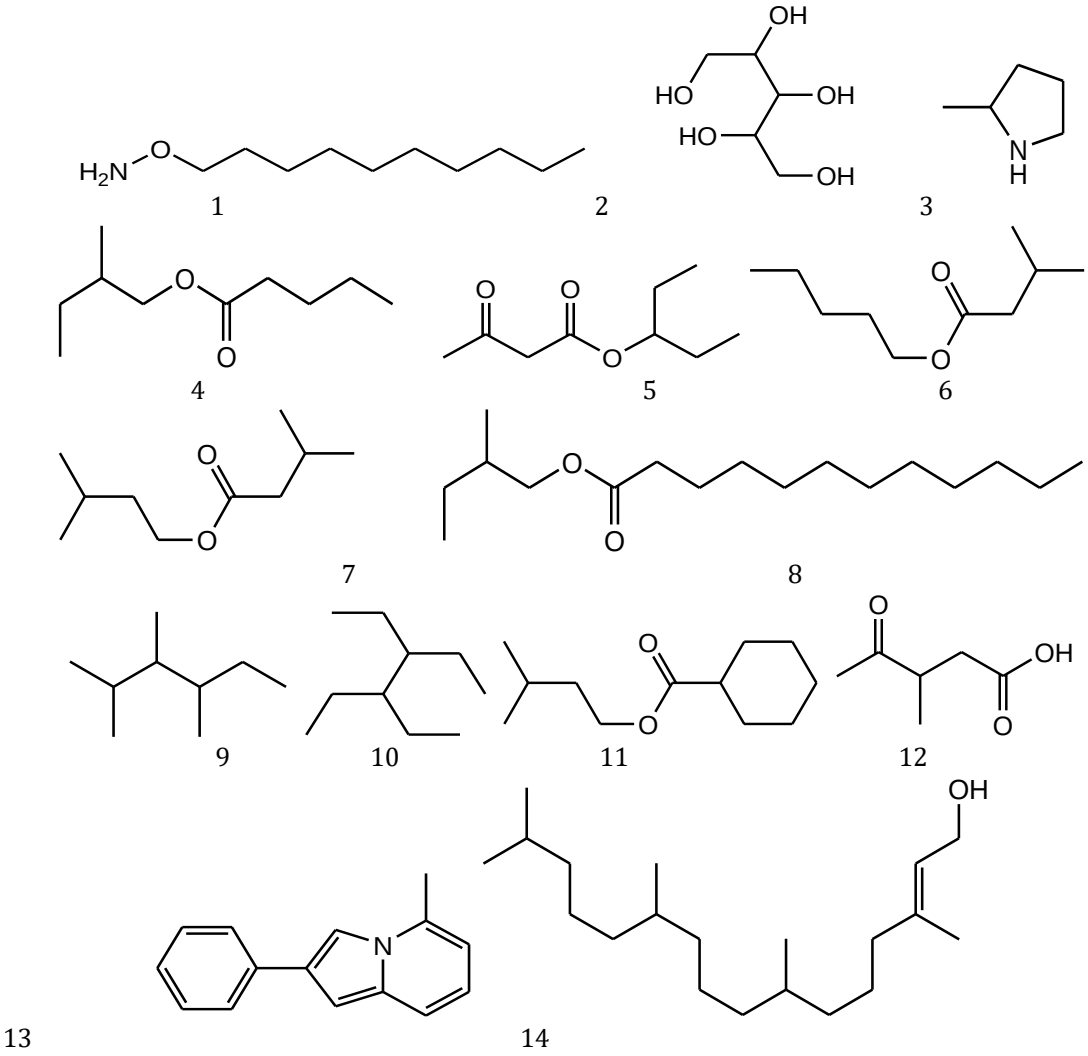


Figure 1a : Structures of secondary metabolites identified in ethyl acetate extract of A. rabaiensis leaf: O-decyl hydroxylamine (1), DL-Arabinitol / (2R,4R)-pentane-1,2,3,4,5-pentol (2), 2-Methylpyrrolidine (3), 2-methylbutyl pentanoate (4), pentan-3-yl 3-oxobutanoate (5), pentyl 3-methyl butanoate (6), 3-methylbutyl 3-methylbutanoate (7), 2-methylbutyl dodecanoate (8), 2,3,4-trimethylhexane (9), 3,4-diethylhexane (10), 3-methylbutyl cyclohexane carboxylate (11), 3-methyl-4-oxopentanoic acid (12), 5-methyl-2-phenylindolizine(13), 3,7,11,15-tetramethylhexadec-2-en-1-ol (14)

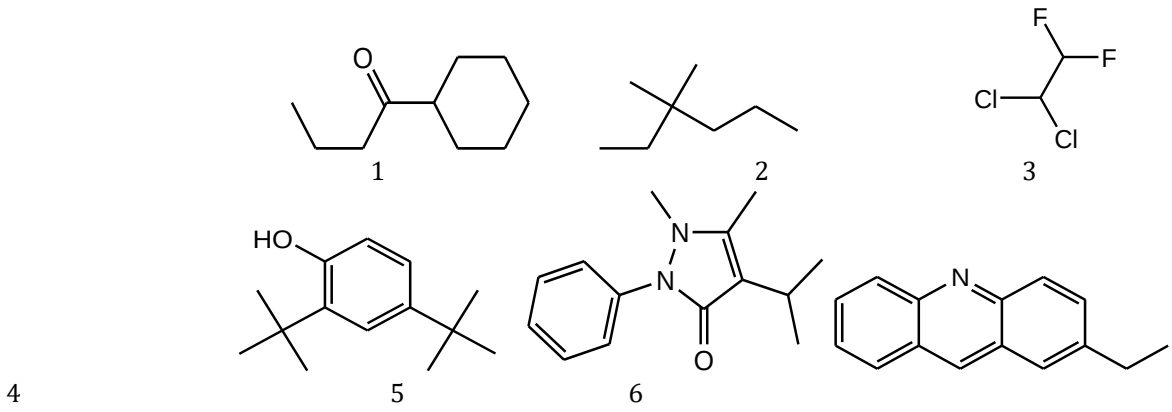


Figure 1b: Structures of secondary metabolites identified in leaf chloroform extract of A. rabaiensis leaf: 1-cyclohexylbutan-1-one (1), 3, 3-dimethylhexane (2), 1, 1-dichloro-2, 2-difluoroethane (3), 2, 4-Di-tert-butylphenol (4), 1, 5-dimethyl-2-phenyl-4-propan-2-ylpyrazol-3-one (5), 2-ethylacridine (6)

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Discussion

The GC-MS analysis of crude extracts of *A. rabaiensis* leaf through sequential extraction revealed the presence of various bioactive compounds. No primary compound(s) were obtained from both crude extracts of ethyl acetate and chloroform of *A. rabaiensis*. However, the phytoconstituents from *A. rabaiensis* are biologically active with different pharmacological activities (Tables 1 and 2).

The GC-MS analysis of *A. rabaiensis* ethyl acetate extract identified fourteen phytocompounds as depicted in Table 1 with their structures in Fig. 1a. Among others, the O-decyl hydroxylamine extracted from *A. rabaiensis* is comparable to that from Androsace foliosa with flavour enhancer associated with aroma as observed in initial stages of fortifying cheese; however, in the final stage, the aroma diminishes, thus used as an effective compound for assessment of cheese quality [36, 37]. The O- decyl hydroxylamine from the cuticle of Camponotus fellah and seed of *Cuminum cyminum* is used as a potent broad spectrum antibacterial and insecticidal agent against *Sitophilus zamias* and *Tribolium castaneum* [38]. Other uses of this compound are as a spice, an antioxidant, and a photosensitive material. In A. foliosa seed extract, the O-decyl hydroxylamine is a major compound that was revealed to possess antimicrobial activity against Gram-positive bacteria *Streptococcus pneumonia* and *Bacillus subtilis*, Gram-negatives *Pseudomonas aeruginosa* and *Escherichia coli* and as an antifungal agent against *Aspergillus fumigatus* and *Candida albicans* [37].

The volatile esters identified from *A. rabaiensis* are known for their contribution to the aroma in Jackfruit (*Artocarpus heterophyllus*), baby banana fruit (*Musa acuminata* AA Simmond cv. *Bocadillo*), sea buckthorns (*Hippophae rhamnoides* L.) are pentyl 3-methylbutanoate and 3-methylbutyl 3-methylbutanoate [39, 40]. The aroma provided by these bioactive is essential in improving food quality in the food processing industry and enhancing organoleptic properties to consumers. During the sterilization of grape juice, 3-methyl-4-oxopentanoic acid constitutes more volatile compounds by 61.57% of the total volatile compounds that demonstrated antimicrobial properties [41]. The phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol), a precursor for manufacturing vitamins E and K, is a potential antidepressant for central nervous system diseases in humans and animals [42]. A similar compound has been reported to have anticancer and antimicrobial activities [43].

Six phytochemical compounds were identified from the chloroform extract of *A. rabaiensis* leaf. The compounds exhibited different pharmacological activities, as shown in Table 2. The 2,4-di-tert-butylphenol identified from *A. rabaiensis* is an active sweet potato component that works against oxidative stress and cytotoxicity in a pheochromocytoma cell line (PC 12) extracted from rats [44, 45]. A pheochromocytoma cell line is a tool for genes that regulate cell growth and differentiation [46]. Others are 1,5-dimethyl-2-phenyl-4-propan-2-ylpyrazol-3-one, demonstrating antipyretic, anti-inflammatory, and analgesic properties as reported by Tighadouini [47].

Despite limited literature on *A. rabaiensis*, their crude extracts revealed effectiveness against pathogens. A study by Mkangara and Mpenda [48] exhibited the minimum inhibitory concentration (MIC) of 0.390 mg/mL in *A. rabaiensis* methanolic and ethyl acetate extracts against Salmonella gallinarum. In a similar study, the minimum bactericidal concentrations (MBCs) ranged from 1.563 to 3.125 mg/mL for the same extracts. The MIC and MBC demonstrated during the antimicrobial activity were verified for toxicity assay to assess the efficacy of *A. rabaiensis* in treatment against pathogens without causing undesirable health effects to an organism. According to Mkangara et al. [49], the *A. rabaiensis* leaf has been proven safe once administered to a female albino mouse of eight weeks old up to 3000 mg/Kg. From this observation, *A. rabaiensis* is in category 5 of the global harmonising system (GHS) of the classification and labelling of chemicals [50]. Category 5 is the lowest toxicity class with LD50 ranging from 2000 mg/Kg < LD50 < 5000 mg/Kg during acute toxicity study without considering repeated exposure. However, the concentrations of bioactive compounds in plants vary with the geographical conditions of the area where a plant has been grown and harvested. These characteristics include soil chemistry, humidity, temperature, and amount of rainfall. Therefore, investigating the A. rabaiensis from other regions of Kenya and Somalia remains significant for exploring the levels of bioactive compounds in A. rabaiensis leaf that will be assessed against human and animal-related ailments.

Conclusion

The identified phytocompounds from *A. rabaiensis* and their pharmacological properties support the plant's ethnomedical and ethnoveterinary applications. The bioactive compounds by GC-MS serve as the basis for further biological and pharmacological investigations. Thus, the study warrants the isolation of pure compounds from A. rabaiensis for in vitro and in vivo investigations against animal and human pathogenic microbes to support drug development from bioproducts.

Abbreviation

GC-MS: gas chromatograph mass spectrometry

GHS: global harmonising system

MBC: minimum bactericidal concentration

MIC: minimum inhibitory concentration

LD: lethal dose

PC12: pheochromocytoma cell line 12

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Conflict of interest

I declare that there is no conflict of interest

Consent for publications

An author approved the final manuscript for publication .

Availability of data and material

Data are available on request from the author.

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