

GC-MS Profiling and *In-vitro* Antidiabetic Activity of Leaf Extracts of *Ravenala madagascariensis* Sonn.

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Abstract

This study aimed to document the GC-MS profile and *in-vitro* α -amylase and α -glucosidase inhibitory activity of *Ravenala madagascariensis* Sonn. The shade-dried and powdered plant material after successive Soxhlet extraction with n-hexane and ethyl acetate was subjected to gas chromatography-mass spectrometry (GC-MS) analysis, followed by α -amylase and α -glucosidase inhibition using standard acarbose. Various bioactive compounds, including phytol, tritetracontane, squalene, tetratriacontane, γ -tocopherol and its ester derivative, with peak area percentages of 5.05, 5.94, 4.15, 14.96, 11.41, 43.93%, respectively, were recorded in n-hexane extract. The ethyl acetate extract also showed compounds including 2-Pentanone, 4-hydroxy-4-methyl derivative, 7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione, phytol, (+)- γ -Tocopherol, O-methyl derivative, Tetratriacontane and γ -Tocopherol with a peak area percentage of 53.25, 2.08, 2.20, 24.64, 4.77, 2.65% respectively. Moreover, *in-vitro* analysis indicated that the ethyl acetate extract exhibited stronger α -amylase inhibitory activity (IC_{50} = 91.75 μ g/mL) compared to the n-hexane extract (IC_{50} = 229.78 μ g/mL), with potency approaching that of the standard drug acarbose (IC_{50} = 78.72 μ g/mL). In the α -glucosidase inhibition assay, the

n-hexane and ethyl acetate extracts showed IC_{50} values of 59.40 μ g/mL and 54.40 μ g/mL, respectively, which were comparable to that of the standard metformin (IC_{50} = 55.49 μ g/mL). In summary, the ethyl acetate leaf extract of *Ravenala madagascariensis* Sonn. exhibited potent α -amylase inhibitory activity, indicating its potential to reduce postprandial glucose levels, while both extracts showed significant α -glucosidase inhibition, aiding in delayed carbohydrate digestion and glucose absorption. The presence of diverse volatile phytoconstituents further supports their biological activity, highlighting the plant's promise for development into an effective antidiabetic formulation.

Key words: Anti-diabetic; α -amylase; GC-MS; *Ravenala madagascariensis* Sonn.

Introduction

Diabetes mellitus is a metabolic disorder marked by elevated blood glucose levels resulting from reduced insulin secretion, impaired insulin action, or both. In 2013, an estimated 382 million people worldwide were reported to have diabetes, and it is projected to rise to 592 million by 2035 (1). Chronic hyperglycemia, resulting from insufficient insulin activity, disrupts normal carbohydrate, lipid, and protein metabolism (2). Type 1 diabetes, also called insulin-dependent diabetes, is an autoimmune disorder in

which pancreatic β cells are destroyed, leading to a complete lack of insulin (3). In contrast, Type 2 diabetes develops due to a combination of insulin resistance and impaired insulin secretion, which together disturb glucose homeostasis and reduce the body's ability to regulate blood sugar effectively (4). For centuries, plant-based preparations have formed the cornerstone of medical treatment, and their relevance continues even in modern medicine. Many individuals still rely on herbal remedies to manage conditions such as pain, fever, anxiety, asthma, gastritis, diabetes, and other health disorders (5). According to a report published by *The News*, Pakistan ranks third globally in the prevalence of diabetes, following China and India (6). Several factors contribute to the development of type II diabetes mellitus, including hyperlipidemia, obesity, hypertension, physical inactivity, advancing age, a history of gestational diabetes, cultural variations, and genetic predisposition (7, 8). Despite advances in medical science, there is no definitive cure for diabetes. Consequently, herbal medicines are increasingly being explored as complementary therapeutic options for diabetes management (9). In this context, traditional medicinal practices may offer natural and promising approaches for addressing diabetic conditions (10). Diabetes can be treated with oral drugs, including sulfonylureas, meglitinides, biguanides, thiazolidinedione, and α -glucosidase inhibitor, of which acarbose is one example (11). But these medications are usually linked to side effects, among them are hypoglycemia, gastrointestinal and cardiovascular disorders, bone fractures, and hepatotoxicity, and they can be very costly (12). Because of these, plant medicines are seen to be gaining popularity as effective and more affordable alternatives. Herbal plant medicines have been employed traditionally because of their valuable biologically active compounds, which are manifested by the existence of phenols, alkaloids, flavonoids, glycosides, tannins, and terpenoids (13, 14). Advances in plant genetics further amplify the benefits of plant medicines, where their valuable metabolic properties are further ele-

vated because of the increased production of their beneficial compounds (15). Among these are plants like *Gynura procumbens*, *Terminalia arjuna*, *Azadirachta indica*, and *Dioscorea alata*, which show significant properties as antidiabetic agents (16-19).

Ravenala madagascariensis Sonn. (Strelitziaceae), commonly known as the Traveller's tree, is native to Madagascar and is characterized by a distinctive fan-shaped arrangement of large, alternately positioned leaves. Traditionally, the plant has been used for the management of diabetes and kidney stones (20, 21), and its seeds have been reported to possess antiseptic properties (22). Despite its extensive ethnomedicinal use, there is a lack of systematic scientific evidence evaluating its antidiabetic potential, particularly in terms of enzyme-based mechanisms such as α -amylase and α -glucosidase inhibition, as well as limited information on its phytochemical profile. Therefore, the present study was undertaken to investigate the in vitro enzyme inhibitory activity of *R. madagascariensis* leaf extracts to assess their potential in reducing postprandial blood glucose levels, along with the identification of bioactive constituents using GC-MS analysis.

Materials and Methods:

Chemicals and reagents

Alpha-amylase (from porcine pancreas), soluble starch, and 3, 5-dinitrosalicylic acid (DNS) were obtained from certified suppliers. All other chemicals and reagents used in the study were of analytical grade.

Collection and authentication of plant material

Leaves of *Ravenala madagascariensis* Sonn. (500 gms) were collected from Guduvancheri, Tamil Nadu during December 2022. Fully grown leaves were collected in a fine dry weather and are stacked loosely in a vertical position in a shed for 3 weeks. The plant was identified and authenticated by Siddha Central Research Institute, Arumbakkam, Chennai. The shade dried leaves were coarsely powdered

and used for further studies. A voucher specimen has been reserved in the Department of Pharmacognosy, SRM College of Pharmacy, Chennai.

Extract preparation

Extraction yields the metabolites into the extracting solvent. The solubility of the metabolites depends on their polarity. The extraction was performed using successive soxhlet extraction of coarsely powdered leaves of *Ravenala madagascariensis* Sonn, using n-hexane and ethyl acetate as extracting solvent. Further, the extract was concentrated by distilling the solvent and the percentage yield was calculated (23).

Estimation of total phenolic content

The total phenolic content of the n-hexane and ethyl acetate extracts was determined using the Folin–Ciocalteu method. Gallic acid was used as the standard reference compound. A stock solution of gallic acid (1 mg/mL) was prepared by dissolving 25 mg of gallic acid in 25 mL of methanol. Similarly, 10 mg of each test extract was dissolved in 10 mL of methanol to obtain the sample solutions. A series of gallic acid standard solutions with concentrations of 12.5, 25, 50, 100, and 200 µg/mL was prepared. For the assay, 1 mL of each standard or sample solution was transferred into a 25 mL volumetric flask, followed by the addition of 9 mL of distilled water. The same procedure was followed for the blank. Subsequently, 1 mL of Folin–Ciocalteu reagent and 10 mL of 7% sodium carbonate solution were added to each flask, and the final volume was adjusted to 25 mL with distilled water. The reaction mixtures were incubated at room temperature for 90 minutes, after which the absorbance was measured at 750 nm using a UV–Visible spectrophotometer (24, 25).

Gas chromatography – mass spectrometry (GC-MS)

The gas chromatography-mass spectrometry (GC-MS) analysis was conducted on an Agilent 78908-5977A auto-sampler using

a HP-SMS 5% phenyl methyl silox capillary column (30 mx0.25 mmx0.25 pm) which was coupled to an Agilent 5977A MS detector. About 1 µL sample was injected into GC-MS using splitless mode. At a purge flow of 1ml/min helium was employed as carrier gas and the injection temperature was maintained at 280 °C. The column operation was initiated with the oven held at 50 °C for 2 min, increased to 270°C at a rate of 5 °C/min and held for 10 min. All data were obtained using full-scan mass spectra collected within the scan range of 40-600 amu and were compared with the NIST repository, 2011. In gas chromatography–mass spectrometry (GC–MS), compounds separated by the gas chromatograph are transferred into the mass spectrometer through a heated interface to keep them in the gas phase. Inside the ion source, the compounds are usually ionized using electron ionization (EI at 70 eV), which breaks molecules into predictable fragments that provide useful structural information. These charged fragments are then guided into a mass analyzer, most often a quadrupole, where they are separated based on their mass-to-charge ratio (m/z). The ions are detected to produce a mass spectrum for each chromatographic peak. Compounds are identified and tabulated in Table 2 and 3. By matching their fragmentation patterns and retention times with reference libraries or standards, and their amounts are determined by measuring the area of selected ion peaks (26).

In-vitro anti diabetic activity studies

Alpha-amylase activity

The alpha-amylase inhibitory activity of the n-hexane and ethyl acetate extracts of *Ravenala madagascariensis* Sonn., were assessed. The reaction mixture consisted of 50 µL of 100 mM phosphate buffer (pH 6.8), 10 µL of α-amylase solution (20 U/mL), and 20 µL of the extracts at varying concentrations ranging from 200µg/mL to 50 µg/mL. The mixture was pre-incubated. After pre-incubation at 37 °C for 20 minutes, 20 µL of 1% soluble starch solution (prepared in 100 mM phosphate buffer, pH 6.8) was added as the substrate, and the reaction

mixture was further incubated at 37 °C for 30 minutes. The enzymatic reaction was terminated by adding 100 µL of DNS reagent, followed by boiling the plate in a water bath for 10 minutes for colour development. After cooling, the absorbance was recorded at 540 nm using a micro plate reader (27-30). All experiments were performed in triplicate. The percentage inhibition of α-amylase activity was calculated using the following formula,

Alpha-glucosidase activity

The α-glucosidase inhibitory activity will be measured as described by Adisakwattana S et al., 2010 (31). Briefly, a volume of 60 µl of ethyl acetate and n-hexane extract (500, 250, 100, 50 and 10 µg/ml) and 50 µl of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.2 U/ml) will be incubated in 96 well plates at 37°C for 30 min. After pre-incubation, 50 µl of 5 Mm p-nitrophenyl –α –D -glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) will be added to each well and incubated at 37°C for another 20 min. Then the reaction will be stopped by adding 160 µl of 0.2 M NaCO₃ into each well, and absorbance reading (A) will

be recorded at 405 nm by micro plate reader and compared to a control which had 60 µl of buffer solution in place of the of the extract. For blank enzyme solution will be replaced by buffer solution and absorbance will be recorded. The α-glucosidase inhibitory activity is expressed as inhibition % and will be calculated using the following formula,

Results and Discussion

Medicinal plants play a crucial role in the effective management of diabetes mellitus, particularly in developing countries with limited healthcare resources. The percentage yield of n-hexane and ethyl acetate extract after successive soxhlet extraction of leaves of *Ravenala madagascariensis* Sonn. was determined and tabulated in Table 1. Among the two extracts, the ethyl acetate extract showed a higher percentage yield of 4.38 %w/w compared to the n-hexane extract (0.75% w/w). Moreover, the total phenolic content of n-hexane and ethyl acetate extract of leaves of *Ravenala madagascariensis* Sonn was found to be 51.18 ± 9.33 and 152.24 ± 27.44 mg GAU/g of the extract respectively.

Table 1: The percentage yield of n- hexane and ethyl acetate extracts of leaves of *Ravenala madagascariensis*.Sonn.

S. No.	Extract	Method of Extraction	Solvent Used	Physical Nature	Colour	Yield (%W/W)
1	Leaves of <i>Ravenala madagascariensis</i> Sonn	Successive soxhlet extraction	n- hexane	Liquid	Light green	0.75%w/w
			Ethyl acetate	Liquid	Dark green	4.38%w/w

The results of GC-MS analysis of the n-hexane and ethyl acetate extract were tabulated in Table 3 and Table 4 respectively. The GC-MS analysis revealed the presence of a diverse range of bioactive phytochemicals in both the n-hexane and ethyl acetate extracts of *Ravenala madagascariensis* Sonn. (Figure.1 & 2). The n-hexane extract identified 21 compounds, with O-methyl γ-Tocopherol, tetratriacontane, tritetracotane, squalene, and phytol being the major constituents. Notably, γ-Tocopherol derivative showed the highest peak area percentage (43.93%), followed by tetratriacontane (14.96%) and γ-Tocopherol (11.41%). These compounds

are known for their antioxidant and therapeutic properties, which may contribute to the biological activity.

Similarly, the ethyl acetate extract identified 20 compounds, with 2-Pentanone, 4-hydroxy-4-methyl derivative (53.25%), phytol (24.64%), and (+)-γ-Tocopherol O-methyl derivative (4.77%) being predominant. Many of these identified phytochemicals, such as γ-Tocopherol and phytol, have been previously reported for their anti-inflammatory, antioxidant, and antidiabetic potential, supporting the relevance of these extracts in pharmacological applications.

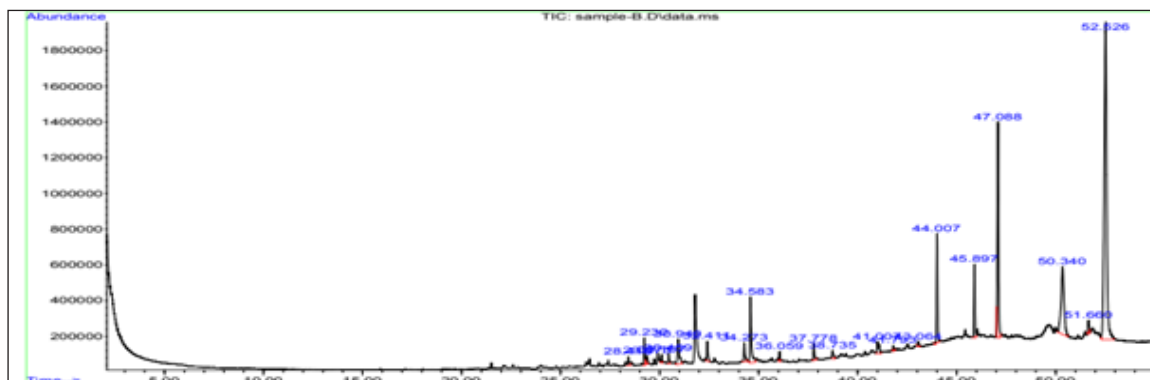


Figure 1: GC-MS chromatogram of bioactive compounds present in n-hexane extract of leaves of *Ravenala madagascariensis* Sonn.

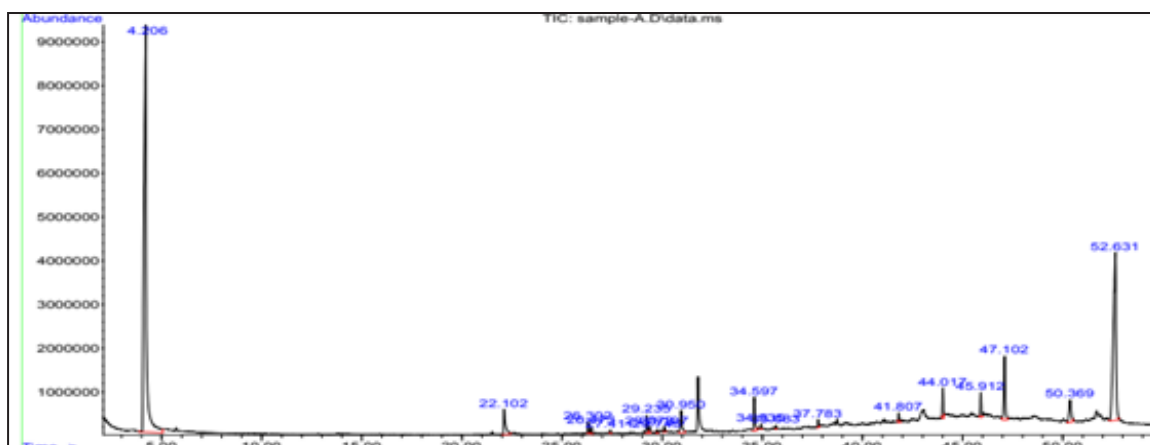


Figure 2: GC-MS chromatogram of bioactive compounds present in ethyl acetate extract of leaves of *Ravenala madagascariensis* Sonn.

Table 2: Phytocompounds identified in GC-MS fingerprint of n-hexane extract of leaves of *Ravenala madagascariensis* Sonn.

S.No	Retention Time	Name of the Compounds	Molecular Formula	Molecular Weight	Peak Area Percentage
1	28.416	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	240	0.67%
2	29.23	3,7,11,15-Tetramethyl-2-hexadecan-1-ol	$C_{20}H_{40}O$	296	1.52%
3	29.373	1-Hexadecanol-2-methyl-	$C_{17}H_{36}O$	256	0.52%
4	30.107	Cyclopropanenonanoic acid, 2-[(2-butylcyclopropyl)methyl]-, methyl ester	$C_{21}H_{38}O_2$	322	0.48%
5	30.459	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	240	0.63%
6	30.94	7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	2.53%
7	32.411	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	240	1.24%

8	34.273	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	240	1.29%
9	34.583	Phytol	$C_{20}H_{40}O$	296	5.05%
10	36.059	tert-Hexadecanethiol	$C_{16}H_{34}S$	258	0.56%
11	37.778	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568	1.16%
12	38.735	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	0.65%
13	41.007	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326	1.36%
14	41.793	18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetradehydro-16-(hydroxymethyl)-, methyl ester, (15 β ,16E)-	$C_{21}H_{24}N_2O_3$	352	0.38%
15	43.064	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	0.37%
16	44.007	Tritetracontane	$C_{43}H_{88}$	604	5.94%
17	45.897	Squalene	$C_{30}H_{50}$	410	4.15%
18	47.088	Tetratriacontane	$C_{34}H_{70}$	478	14.96%
19	50.34	γ -Tocopherol	$C_{28}H_{48}O_2$	416	11.41%
20	51.66	Oleic acid, eicosyl ester	$C_{38}H_{74}O_2$	562	1.21%
21	52.526	(+)- γ -Tocopherol, O-methyl-	$C_{29}H_{50}O_2$	430	43.93%

Table 3: Phytochemicals identified in GC-MS fingerprint of ethyl acetate extract of leaves of *Ravenala madagascariensis* Sonn.

S.No	Retention Time	Name of the Compounds	Molecular Formula	Molecular Weight	Peak Area Percentage
1	4.206	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	116	53.25%
2	22.102	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	2.76%
3	26.302	2-Dodecen-1-yl(-)succinic anhydride	$C_{16}H_{26}O_3$	266	0.52%
4	26.473	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	240	0.37%
5	27.411	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	240	0.19%
6	29.235	Phytol, acetate	$C_{22}H_{42}O_2$	338	0.95%
7	29.373	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$	256	0.43%
8	29.745	Ethanol, 2-(9-octadecenyl-oxo)-, (Z)-	$C_{20}H_{40}O_2$	312	0.23%
9;	30.107	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.30%

10	30.950	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	2.08%
11	34.597	Phytol	$C_{20}H_{40}O$	296	2.20%
12	34.935	Heptadecane, 9-hexyl-	$C_{23}H_{48}$	324	0.28%
13	35.683	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	0.19%
14	37.783	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568	0.50%
15	41.807	18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetrahydro-16-(hydroxymethyl)-, methyl ester, (15 β ,16E)-	$C_{21}H_{24}N_2O_3$	352	0.64%
16	44.017	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	1.60%
17	45.912	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	$C_{30}H_{52}O$	428	1.45%
18	47.102	Tetratriacontane	$C_{34}H_{70}$	478	4.77%
19	50.369	γ -Tocopherol	$C_{28}H_{48}O_2$	416	2.65%
20	52.631	(+)- γ -Tocopherol, O-methyl-	$C_{29}H_{50}O_2$	430	24.64%

The anti-diabetic activity of n-hexane and ethyl acetate extracts of leaves of *Ravenala madagascariensis* Sonn. were displayed in Figure 3 & 4. The present study evaluated the *in-vitro* antidiabetic potential of *Ravenala madagascariensis* leaf extracts through inhibition of the carbohydrate-digesting enzymes α -amylase and α -glucosidase, which play a crucial role in regulating postprandial blood glucose levels. The results demonstrated that both n-hexane and ethyl acetate extracts exhibited concentration-dependent inhibition of α -amylase activity. Among the tested extracts, the ethyl acetate extract showed significantly higher inhibitory activity (85.65%) at 200 μ g/mL, which was comparable to the standard drug acarbose (87.78%). The IC_{50} value of the ethyl acetate extract (91.75 μ g/mL) further confirmed its notable inhibitory potential, whereas the n-hexane extract exhibited lower activity with an IC_{50} value of 229.78 μ g/

mL. This difference in activity may be attributed to the higher solubility of bioactive phytoconstituents such as flavonoids, phenolics, and other polar compounds in ethyl acetate, which are known to contribute to enzyme inhibition.

Similarly, both extracts demonstrated significant α -glucosidase inhibitory activity, which is an important mechanism for delaying carbohydrate digestion and glucose absorption. At the maximum tested concentration of 500 μ g/mL, the n-hexane extract showed 81.31% inhibition, while the ethyl acetate extract exhibited 83.40% inhibition, compared with the standard metformin (91.66%). The IC_{50} values of the extracts were found to be 54.40 μ g/mL for the ethyl acetate extract and 59.40 μ g/mL for the n-hexane extract, which are close to the standard metformin (55.49 μ g/mL), indicating promising inhibitory potential.

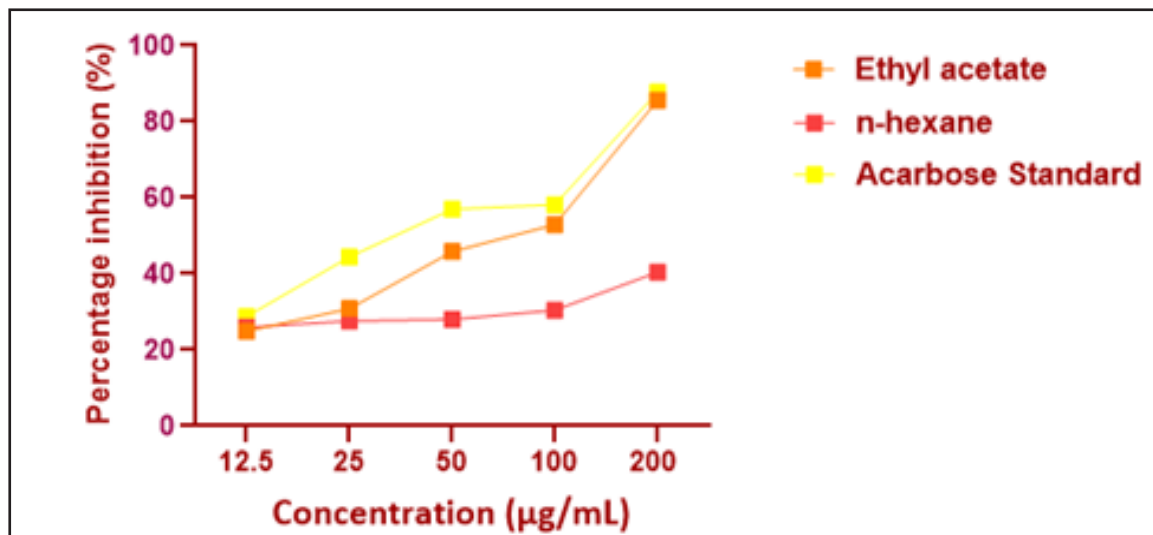


Figure 3: *In-vitro* α -amylase inhibitory effect of n-hexane and ethyl acetate extracts of *Ravenala madagascariensis* Sonn. Data expressed in terms of Mean $\pm$ SEM.

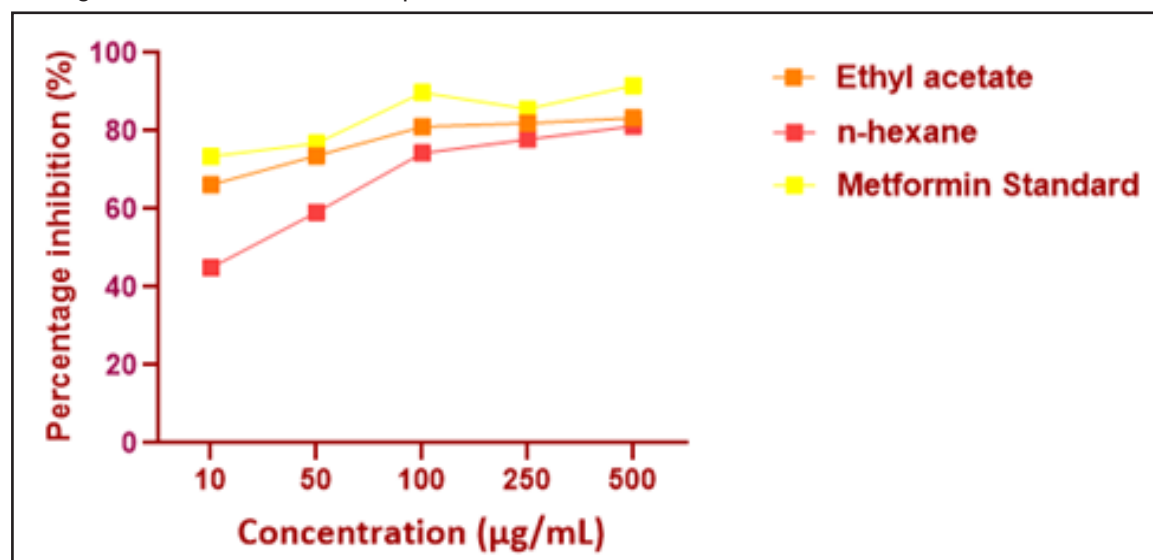


Figure 4: *In-vitro* α -glucosidase inhibitory effect of n-hexane and ethyl acetate extracts of *Ravenala madagascariensis* Sonn. Data expressed in terms of Mean $\pm$ SEM.

Conclusion

The present study demonstrated the *in-vitro* antidiabetic potential of *Ravenala madagascariensis* leaf extracts through significant inhibition of key carbohydrate-digesting enzymes, α -amylase and α -glucosidase. GC-MS analysis revealed diverse phytoconstituents, with

the ethyl acetate extract being particularly rich in bioactive compounds such as γ -tocopherol, squalene, phytol, and long-chain hydrocarbons. Among the tested extracts, the ethyl acetate extract showed superior α -amylase inhibition (85.65%, IC_{50} : 91.75 μ g/mL), comparable to acarbose, while both extracts exhibited strong α -glucosidase inhibition, with IC_{50} values close

to that of metformin. Overall, the findings highlight the promising antidiabetic activity of *Ravenala madagascariensis*, especially the ethyl acetate extract, supporting its potential as a natural source for developing effective antidiabetic agents and validating its traditional use in managing hyperglycemia.

Declaration of Interest

The author declares that they have no known competing financial interests or personal relationships.

Author Contributions

Conceptualization: S.M.K., P.J.K., L.E., S.P.S., K.R.; Methodology: S.M.K., S.S., L.R.B., P.J.K., L.E.; Writing – original draft: S.M.K., S.S., L.R.B., P.J.K., L.E.; Writing – S.M.K., P.J.K., L.E.; review: S.P.S. & K.R.; editing: L.E., S.P.S. & K.R.

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