RESEARCH PAPER

Comprehensive Hr-Lcms-Based Characterization And Anti-Diabetic Assessment Of K.Nemoralis

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ABSTRACT

Diabetes mellitus (DM), which is categorized by abnormally raised Blood Glucose (BG) levels, is an international health crisis. Kyllinga nemoralis is a medicinal plant that has been utilized as a treatment for metabolic disorders, including diabetes; however, its chemical profile and potential anti-diabetic effects have yet to be evaluated comprehensively with modern analytical approaches. The current study assessed the ethanolic extract of K. nemoralis using High-Resolution Liquid Chromatography–Mass Spectrometry (HR-LCMS) to detect its constituents and its potential anti-diabetic and anti-oxidant effects. From the HR-LCMS analysis, nineteen bioactive constituents were identified that belonged to different phytochemical classes, including carbohydrates, phenols, flavonoids, tannins, saponins, alkaloids, steroids, terpenoids, and quinones. The extract also demonstrated concentration-dependent free radical scavenging activity, which confirmed its strong antioxidant potential. In vivo studies in diabetic Wistar rats showed that K.nemoralis extract decreased BG while promoting increased body weight. Moreover, in vitro experiments demonstrated anti-diabetic activity as evidenced by inhibition of α-amylase and α-glucosidase activity by the extract. In histopathological examinations, restoration of pancreatic tissue was noted for the treatment groups. These findings highlight the therapeutic potential of K. nemoralis as a natural source of bioactive compounds for developing plant-derived antidiabetic agents..

Keywords: Kyllinga nemoralis, Anti-diabetic activity, Phytochemical screening, HR-LCMS analysis, ethanolic extract.

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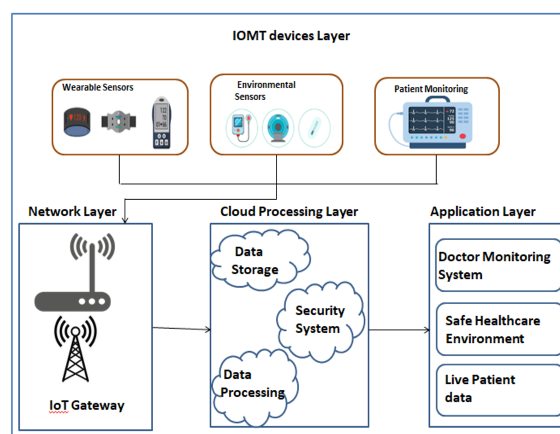
INTRODUCTION

DM is one among the well-known chronic metabolic ailments defined by hyperglycemia, impacting individuals globally. It is strongly associated with difficulties like cardiovascular disease and chronic kidney disease (Baynest, 2015). The DM's treatment currently relies on lifestyle intervention, oral medication, injected drugs, and complementary and alternative treatment (Pang et al., 2019). Many of these medications exhibit significant adverse effects and are incapable of averting the onset of diabetes complications (Chen et al., 2015). Over the previous decades, the DM treatment strategies have improved. Nevertheless, severe adverse effects, including hypoglycemia coma and hepatic and renal problems, were induced by the anti-diabetic drugs (Yedjou et al., 2023). When compared to synthetic drugs, herbal-based drugs, such as those derived from plants, serve as both preventive and therapeutic agents due to their diverse bioactive compounds and lower toxicity and side effects. Conventionally, for treating various ailments, including diabetes, different plants are used (Bhatia et al., 2019). To treat conditions like diabetes and hyperglycemia, members of the plant family Cyperaceae have been used (Kamala et al., 2018). The plant *K.nemoralis* of Cyperaceae has analgesic, antidiabetic, anticancer, anti-oxidant, and



antimalarial properties (Wahab & Rahman, 2022). Numerous phytomolecules, encompassing flavonoids, saponins, alkaloids, terpenes, anthraquinones, coumarins, phenolics, and polysaccharides, are included in *K.nemoralis,* which possesses antidiabetic properties (Swain, 2023). BG level and lipid parameters are mitigated by the plant extract's antidiabetic potential (Rajendiran, 2018). The active principles obtained as of the plants perform via numerous anti-diabetic strategies (Yue et al., 2017).

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(Datta et al., 2022) propounded that *K.nemoralis* illustrated α-amylase inhibition of IC_{50}=72.62 ± 0.01[b] and α-glucosidase inhibition of IC_{50}=236.90 ± 0.003[a]. Furthermore, the researchers found that the plant exhibited considerable antioxidant, anti-inflammatory, and anti-diabetic capacity, attributed in part to a wide range of bioactive metabolites, extensive flavonoid diversity in particular. The alcoholic extract of K. nemoralis aerial parts was shown to reduce ferric ions in a concentration-dependent manner (Rajagopal et al, 2016), again suggesting possible antioxidant activity. The availability of flavonoids, phenols, tannins, steroids, saponins, and terpenoids was exposed by the *K.nemoralis'* phytochemical screening, with enormous phenols and tannins. 19 compounds were detected by GC-MS analysis, illustrating antibacterial, antifungal, and anticancer activities (Bhavani et al., 2025). The phytochemicals and GC-MS analysis of *K.nemoralis* were investigated by another author (Chithra & Nair, 2021). Here, polyphenol, tannin, and flavonoids were found to be high in the whole plant. The presence of a high amount (63.15%) of terpenoid compounds was unveiled by the analytical investigation of the plant rhizome's needed oil.

As stated by (Prathyusha & Velraj, 2022), the inhibition slowed the glucose absorption rate, thus lowering BG levels. The outcomes of HR-LCMS indicated that Michelia champaca contained 15 phytochemical compounds. (Nallapaty et al., 2024) performed HR-LCMS analysis to reveal the phytochemicals and anti-diabetic activity in another plant named *Chrozophora rottleri*. As per the result, the analysis revealed 31 phytochemicals, such as 14 flavonoids, 7 glycosides, 3 phenols, carbohydrates, fatty acids, and alkaloids. As per the *In-silico* docking simulations, the presence of phyto-chemicals like cryptochlorogenic, aureusidin, remikiren, and genistein 8-C-glucoside showed a stronger binding affinity to glucose-regulating drug targets than standard anti-diabetic medications. Likewise, (Kavitha & Kumudha, 2024) examined the antidiabetic activity of hydro-ethanolic extracts of *Amphiroa Fragilissima* (HEEAF) and screened the phytochemicals using HR-LCMS. The ethanolic extract of *K.nemoralis* exhibited strong enzyme-suppressing activity on α-amylase and α-glucosidase, yielding a stronger inhibitory effect, respectively. These outcomes were evaluated in relation to the inhibitory profiles of standard antidiabetic agents, acarbose and voglibose.

Numerous therapeutic approaches are available due to major advances in the pharmaceutical industry. Side effects are often caused by traditional diabetes treatments, necessitating natural alternatives like *K.nemoralis* as an anti-diabetic agent. There were studies concentrating on the anti-diabetic activities of various medicinal plants, including *K.nemoralis*. GC-MS analysis was utilized by numerous researchers for the identification of bioactive compounds. As per the literature, no studies have examined the bioactive compounds of *K.nemoralis* using HR-LCMS analysis. To the researcher's knowledge, no studies integrated laboratory-based and animal analysis of the anti-diabetic activity of *K. nemoralis*. Therefore, this investigation intends to analyze the phytochemical profile of *K. nemoralis* using HR-LCMS to identify active compounds. It also evaluates the plant extracts in laboratory-based and animal anti-diabetic effects to determine their ability as a diabetes treatment. The following research objectives are developed to clarify the aim further and support the research.

To perform phytochemical screening along with quantitative valuation of total phenolic and flavonoid constituents of *K.nemoralis*, establishing their association with potential anti-diabetic effects.

To separate the bioactive compounds from the *K.nemoralis* plant extracts utilizing HR-LCMS analysis.

To validate the prepared plant extracts' anti-oxidant potential using ABTS, DPPH, FRAP, and Hydrogen peroxide (H_2O_2) RS assays.

To investigate the antidiabetic effect through in vitro enzyme inhibition studies with respect to glycogenase (α-amylase) and Alpha-glucosidase, and to confirm its therapeutic efficacy through in vivo histopathological study of albino Wistar rats.

The latter part of the research is divided into a few sections: Section 2 defines the materials as well as methodologies utilized in this study. The outcomes are analyzed and given in Section 3. In Section 4, the discussion is provided by comparing the present study's outcomes with existing studies. Lastly, the study concludes with a summary of findings, their drawbacks, and future scopes.

2. MATERIALS AND METHODS

2.1. Sample Collection

K.nemoralis were gathered from diverse localities in India, and the Botanical Survey of India verified its identification. The solvents and reagents required for the extraction process were purchased from the chemical suppliers.

2.2. Plant Material and Extraction

The collected plant was cleaned by tap water for 2-3 times and then by distilled water to filter out the dirt particles as well as soil. It was then dried in the shade as well as stored. The samples were finely powdered and fed to Soxhlet extraction utilizing C_2H_5OH. 20g of powdered plant samples with C_2H_5OH (250ml) was extracted for 6 hours. For further studies, the plant extracts were cleaned and then stored in the fridge.

2.3. Qualitative Phytochemical Screening

It is carried out for detecting the availability or absence of numerous classes of natural compounds. For the entire extracts, phytochemical investigations were done utilizing standard methods. In Table 1, the methods for identifying the phytochemical ingredients in the extract are exhibited.

Table 1: Methods to identify phytochemical constituents (Patil & Jain, 2019; Matlala et al., 2024; Vishwakarma & Shrivastava, 2025)

S. No	Phytochemical	Test (Method & Principle)	Observation / Inference

1	Carbohydrates	• Molisch's test – Extract (5 mL) mixed with alcoholic α-naphthol and conc. H_2SO_4. Violet/purple ring formed. • Fehling's test – The extract was acid-treated, neutralized, and reacted with Fehling's A and B; • Benedict's test – The filtrate was heated with Benedict's reagent, producing an orange-red color.	Violet ring / Brick-red / Orange-red precipitates
2	Glycosides	Modified Borntrager's reaction – Extract heated with ferric chloride, cooled, shaken with benzene and ammonia; rose-pink colour in ammoniacal layer.	Rose-pink colour
3	Proteins	• Biuret reaction – NaOH + $CuSO_4$ → violet-pink complex. • Xanthoproteic reaction – Conc. HNO_3 → yellow colour.	Pink-violet / Yellow
4	Amino acids	Ninhydrin reaction – Boiling with 0.25 % ninhydrin → blue-purple colour.	Blue-purple
5	Phenols	Ferric-chloride reaction – $FeCl_3$ → bluish-black or green shade.	Bluish-black / green
6	Flavonoids	• Alkaline-reagent reaction – NaOH → yellow colour, fading on acidification. • Lead-acetate reaction – Yellow precipitate forms.	Yellow colour / Precipitate
7	Tannins	Gelatin reaction – 1 % gelatin + NaCl → white precipitate.	White precipitate
8	Saponins	• Froth reaction – Persistent foam layer (~1 cm).• Foam reaction – Foam stable ≈ 10 min.	Foam formation
9	Alkaloids	• Mayer's reaction – A cream or pale-yellow precipitate formed upon adding Mayer's reagent, confirming alkaloids. • Dragendorff's reaction – The extract acidified with dilute HCl produced an orange to reddish precipitate upon addition of Dragendorff's reagent, confirming alkaloids. • Wagner's reaction – Addition of Wagner's reagent produced a brown-red precipitate, confirming alkaloids.	Yellow / Red / Brown
10	Steroids	Liebermann–Burchard reaction – Bluish-green colour.	Bluish-green
11	Terpenoids	Salkowski reaction – Yellow colour in the lower chloroform layer.	Yellow (lower layer)
12	Quinones	Conc. H_2SO_4 reaction – Deep red colour.	Red colour

2.4. Quantitative Analysis of Phytochemicals

2.4.1. Estimation of total phenol content

The Total Phenolic Content (TPC) was measured by using a modified Folin-Ciocalteu (FC) assay (Darshana & Hemant, 2024). A series of standard solutions was prepared with gallic acid to achieve a final concentration range of 10 to 50 µg/mL. Subsequently, the dried plant extract was reconstituted in ethanol, and debris was removed by filtering. An amount of dried extract containing gallic acid was added to the Folin-Ciocalteu reagent and sodium carbonate, shaken, and maintained at 41 degrees Celsius for 31 minutes. The absorbance was determined at 765 nm wavelength with a ethanol blank. TPC was measured relative to a gallic acid standard, with results shown as milligrams of gallic acid equivalents per gram of dried extract.

2.4.2. Estimation of TFC

The total level of flavonoid was assessed via the modified calorimetric method (Darshana and Hemant, 2024). The plant extract was added to purified water (0.9 ml) and aluminum chloride (1 ml) solutions. The mixture was kept for 1 hour at room temperature to allow the flavonoid–$AlCl_3$ complex to form (indicated by yellow color development). The total flavonoid level for each sample plant extract was expressed as equivalent to bioflavonoid (quercetin).

2.5. Anti-oxidant Assays

2.5.1. ABTS radical scavenging activity

RS activity was examined by FTIR methodology. In the preparation of the ABTS reagent, equal parts of 7mM ABTS and 2.45mM potassium persulfate produced free radicals that had been incubated in the dark for 12 hours. The assay was performed as follows: 1 ml of diluted ABTS+ was mixed/reacted with 100 ml of the appropriate plant extracts to evaluate antioxidant capacity. Absorbance was determined at 734 nm, using Ascorbic acid (AA) as the standard (Datta et al., 2022).

2.5.2. DPPH Assay

By using stable free DPPH radical, the extract's RS activity was measured (Nallapaty et al., 2024). DPPH ethanolic solution was mixed with the extract. The mixture was then vortexed thoroughly and incubated in the dark at room temperature for 32 min to neutralize the DPPH radical. The absorbance was then measured at 517 nm, and an absorbance reduction indicated RS activity. AA was used as a positive control.

2.5.3. Ferric reducing anti-oxidant power (FRAP)

A test sample solution was made at concentrations of 2.5 μ/mL to 100 μ/mL. The test sample solution was mixed with potassium ferricyanide and phosphoric buffer (Nallapaty et al., 2024) and incubated at 52 °C for 25 minutes. Following incubation, the sample was treated with trichloroacetic acid and centrifuged at 3200 rpm for 12 minutes. The supernatant was decanted from the precipitate, diluted in distilled water, and iron (III) chloride was added. The absorbance of the AA standard was then read at 700 nm.

2.5.4. Hydrogen peroxide (H_2O_2) method

A H_2O_2 solution was prepared at 43 mM in 0.1 M phosphate buffer, adjusted to pH 7.4 (Nallapaty et al., 2024). The extract was added to H_2O_2 solution (0.6 mL) at numerous concentrations in phosphate buffer. The absorbance was gauged at 230 nm.

2.6. Anti-diabetic Assays

2.6.1. In-vitro α-amylase assay

100μl of α-amylase solution (0.1 mg/ml) was combined with different concentrations of ME of *K.nemoralis* in the gamut of 10-100 μg/ml. Acarbose was used as the standard for the method. The control solution was placed in an incubator for one minute at 36°C, and no test sample or standard was added. Following the one-minute incubation, the starch solution (100 μL) was added to the incubation tube, and then incubated again. At the end of the reaction time, which was 1 hour, 10 μL of 1 M HCl was added to each of the test tubes, and a reagent of iodine reagent was added to each of the test tubes, and this was mixed. The absorbance was taken at an optical density of 565 nm (Kavitha & Kumudha, 2024).

2.6.2. In-vitro α-glucosidase inhibition assay

The substrate solution was p-nitrophenyl glucopyranoside (pNPG) prepared in phosphate buffer (100 mM, pH 6.8) (Kavitha & Kumudha, 2024). ME of *K.nemoralis* was incubated with α-glucosidase (200 μl) in various concentrations from 10 to 100 (μg/ml) for 12 minutes. The substrate was prepared in phosphate buffer (400 μl) to begin the reaction that was incubated at 36° C for 18 minutes. After the 18-minute incubation, 1ml of 0.1M sodium carbonate was added to stop the reaction process. Acarbose was utilized as a standard reference, and absorbance was read at 410 nm.

2.7. Calculation of % Inhibition

By utilizing the % scavenging actions at 5 diverse absorptions of the extract, the plant extracts' concentration necessary to scavenge half of the radicals (IC_{50}) was computed. The PI was computed by,

$$\textbf{\textit{inhibition}}\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

2.8. HR-LCMS Analysis

HR-LCMS was employed to find the bioactive compounds available in the ME of *K.nemoralis*. At the Sophisticated Analytical Instrument Facility (SAIF) at IIT Bombay in Powai, Mumbai, HR-LCMS analysis of the sample was performed. By utilizing an Agilent HR-LCMS model G6550A with mass resolution of 0.01%, the selected plant extract's chemical fingerprints were prepared. Chromatographic parting was done on the Hypersil GOLD C18 column. 100μl/min flow rate, 5μl flush out factor, and 8μl injection volume were deployed for the HR-LCMS analytical method. CH_3OH: acetonitrile (95:5) served as mobile phase A, whereas 10mM ammonium acetate in water served as mobile phase B. The Mass Spectrometry (MS) acquisition method used a scanning rate of approximately 50 to 1000 Daltons (m/z) per second. The HR-LCMS was kept running at 250°C and 13 psi/min for 35 minutes. By analogizing the spectrum of unknown components to the known components' spectrum, the HR-LCMS mass spectrum was interpreted.

2.9. In Vivo Anti-Diabetic Activity

2.9.1. Animals used

In the study, Albino Wistar rats were employed under standard laboratory conditions. For the study, both male and female rats with BWs ranging from 200 to 220g were used. After obtaining approval as of the Ethics Committee, all animals used for the study were obtained.

2.9.2. Toxicity evaluation

The albino Wistar rats were given single oral doses of *K.nemoralis* ME (500, 1000, 1700, 1800, and 2000 mg/kg) or Tween 80 (control group) in this acute oral toxicity study. The rats were monitored for mortality and general behavior over 48 hours, with continuous observation for the first four hours, followed by observations at the 6, 24, and 48-hour marks to assess for any toxic effects.

2.9.3. Experimental design

For the antidiabetic study, rats were segmented into three main groups (6 rats each). The treatment protocol for 15 days was as follows:

Group 1: Normal control (received food and water only)

Group 2: Disease control (Carboxyl methyl cellulose suspension orally)

Group 3: Rats treated with ME of *K.nemoralis* (150 mg/kg BW orally administered for 15 days)

2.9.4. Histological study

The rats were sacrificed at the final stage of the experiment, followed by isolating their pancreas. The pancreas was stored in Bruin's solution, and histological slides were prepared and studied under the microscope for any histological changes.

2.10. Statistical Analysis

By using statistical techniques, the data analysis was carried out. The entire tests were done in triplicate. The data were articulated as mean and Standard Error Mean (SEM). Findings were articulated as Average ± Standard Error, and the significance was statistically articulated as p value<0.01.

3. RESULTS

Quantitative and qualitative phytochemical screening was conducted in the present study. Furthermore, using HR-LCMS, bioactive compounds in *K.nemoralis* were identified. The anti-oxidant properties were evaluated, and anti-diabetic properties were examined via in vitro and in vivo studies (Datta et al., 2022; Bhavani et al., 2025; Chithra & Nair, 2021).

3.1. Phytochemical screening

To identify the existence of the phytochemical constituents, the extract was evaluated. The existence or absence of numerous compounds. Table 2 summarizes the phytochemical analysis outcomes, where the '✓' and '×' signs specify the presence and absence of a constituent, respectively.

Table 2: Phytochemical screening using the Soxhlet method

Phytochemical constituents		ME of ***K.nemoralis***
Carbohydrates	Molisch's test	✓
	Fehling reagent test	×
	Benedict's reagent	✓
Glycosides	Modified Borntrager's test	×
Proteins	Biuret test	×
	Xanthoproteic test	×
Amino acids	Ninhydrin test	×
Phenols	Ferric chloride test	✓
Flavonoids	Alkaline sensitivity test	✓
	Lead acetate reaction	✓
Tannins	Gelatin precipitation test	✓
Saponins	Froth test	✓
	Foam stability test	✓
Alkaloids	Mayer's reagent	×
	Dragendorff's test	✓
	Wagners test	×
Steroids	Liberman Buchard's test	✓
Terpenoids	Salkowskis test	✓
Quinones	Concentrated H_2SO_4	✓

As per the present study, no glycosides, proteins, or amino acids were found in the *K.nemoralis* with ME. Mayer's and Wagner's tests indicated an absence of alkaloids in the plant extract, while Dragnedorff's test confirmed the presence of it. Similarly, the presence of carbohydrates was assured by Molisch's and Benedict's tests, whereas Fehling's test recorded the absence of carbohydrates in the plant extract. Hence, the existence of compounds was exhibited by the phytochemical screening.

3.2. Estimation of TFC and TPC

The total phenol as well as flavonoid contents of the ME from *K.nemoralis* were tested. In Table 3, the concentration and absorbance of the calibration curve for TPC are shown.

Table 3: Absorbance readings for total phenolic estimation at 765 nm

Phenolic Absorption (µg/mL)	10	20	30	40	50
Observed Absorbance (at 765 nm)	0.448	0.609	0.721	0.956	1.257

The result showed that as concentration increased, absorbance also increased. At 10, 20, 30, 40, and 50 µg/ml, the absorbances were 0.448, 0.609, 0.721, 0.956, and 1.257, respectively. The absorbance wavelength was found to be 765 nm. As illustrated in Figure 1, the TPC of the extract was articulated as *milligrams of gallic acid equivalents*, derived from the standard calibration curve.

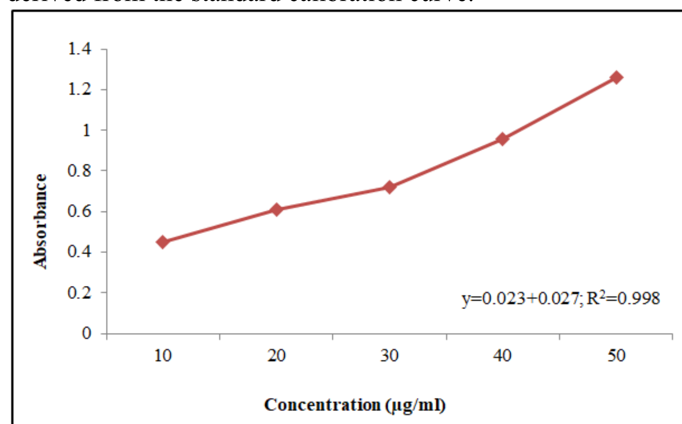

Figure 1: Standard calibration curve of gallic acid

In Table 4, the concentration and absorbance of the calibration curve for TFC are shown.

Table 4: Absorbance readings for total flavonoid quantification at 420 nm

Flavonoid Concentration (µg/mL)	**10**	**20**	**30**	**40**	**50**
Measured Absorbance (at 420 nm)	0.565	0.852	0.987	1.327	1.643

Table 4 revealed that when the concentration increased, the absorbance also increased. The concentration ranged from 10 to 50µg/ml, and the absorbance ranged from 0.565 to 1.643. The absorbance wavelength was found as 420 nm. As shown in Figure 2, the TFCs were articulated as Bioflavonoid.

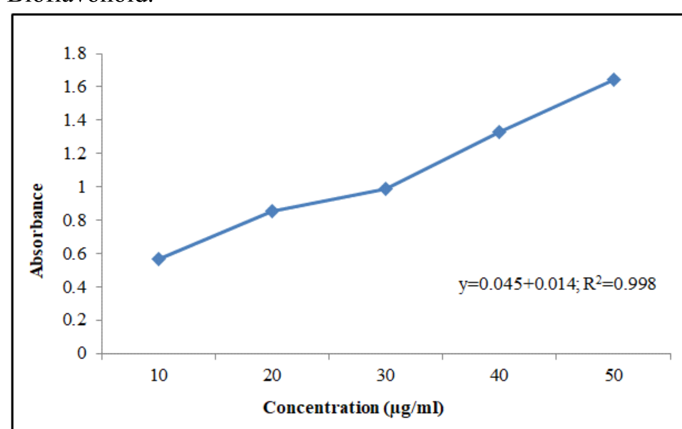

Figure 2: Calibration curve for Bioflavonoid

For determining the total phenol and flavonoid content, the FC technique and aluminium chloride colourimetric assay were utilized, respectively. Table 5 shows the total phenol as well as flavonoid content.

Table 5: Quantification of total phenolic and flavonoid concentrations in the ethanolic extract of *K.nemoralis*

Biochemical Parameter	Concentration in ME of *K.nemoralis*

TPC (mg GAE per g dry extract)	92.26 ± 0.16
Total flavonoid compounds (mg QE per g dry extract)	19.87 ± 0.16

As per the result, the total phenol and flavonoid content obtained for the ME of *Kyllinga nemorais* were 92.26±0.16 and 19.87±0.16, respectively.

3.3. Identification of bio-active compounds by HR-LCMS

For identifying various compounds, the HR-LCMS analysis was employed. By analogizing with available standards, the individual compounds were detected. The detection was performed on the plant material with a ME. In Table 6, the compounds obtained from the plant material are given.

Table 6: Compounds of *K.nemoralis* with ethanolic extract

S. No	Name of the compound	Formula	Molecular weight	Mass error (ppm)	RT (min)	Peak
1	3-(Prop-2-enoyloxy)tetradecane	$C_{17}H_{32}O_2$	268.435	0.99	0.87	10.0
2	1-chloro-1-fluoroethane	C_2H_4ClF	185.967	1.20	5.06	9.26
3	2-butanone	C_4H_8O	72.106	1.20	6.11	9.75
4	3-pentanone	$C_5H_{10}O$	86.132	0.65	9.45	7.42
5	7-methylbicyclo [3.2.0]hept-3-ene-2-one	$C_8H_{10}O$	122.164	0.60	11.34	13.56
6	2-methyl butanoic acid methyl	$C_6H_{12}O_2$	116.158	1.74	15.02	9.35
7	1,3-dichlorobenzene	$C_6H_4Cl_2$	147.002	0.88	16.01	22.24
8	Cycloheptasiloxane, tetradcamethyl	$C_{14}H_{42}O_7Si_7$	519.078	0.82	19.6	31.35
9	p-Hydroxybenzoicacid	$C_7H_6O_3$	138.121	0.93	19.8	23.44
10	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.237	2.61	25.01	22.19
11	Cyclooctasiloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	593.232	0.50	26.10	35.49
12	Nonane 5- (2-methyl propyl)	$C_{13}H_{28}$	184.361	-0.19	29.01	41.35
13	Heptasiloxane, hexadecamethyl	$C_{16}H_{48}O_6Si_7$	533.147	0.53	30.11	71.55
14	1,2-Benzene dicarboxylic acid, bis (2-methylpropyl)	$C_{16}H_{22}O_4$	278.343	1.16	32.54	98.18
15	Tetradecane 2,6,10 trimethyl	$C_{17}H_{36}$	240.468	2.15	34.80	32.66
16	13,16 Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.472	-4.28	36.11	39.20
17	Cyclo 3octa siloxane hexadecamethyl	$C_{16}H_{48}O_8Si_8$	593.232	4.39	39.67	51.28
18	Cyclononasiloxane octadecamethyl	$C_{18}H_{54}O_9Si_9$	667.385	1.04	40.12	43.34
19	Tetracosamethyl cyclododeca siloxane	$C_{24}H_{72}O_{12}Si_{12}$	889.847	0.70	45.01	8.45

Table 6 summarizes the tentative compounds that are identified from the extracts along with their molecular weight, retention time, peak area, and molecular formula. 19 compounds were found in the *K.nemoralis* with the ME. Each compound was characterized based on its experimental mass values, with a matched isotopic pattern over 75% and a mass deviation lower than 5 ppm. 1,2-Benzene dicarboxylic acid, bis(2-methylpropyl) produced the largest peak (98.18 intensity), with a retention time of 32.54 minutes. In Figure 3, the chromatogram of the HR-LCMS analysis is shown.

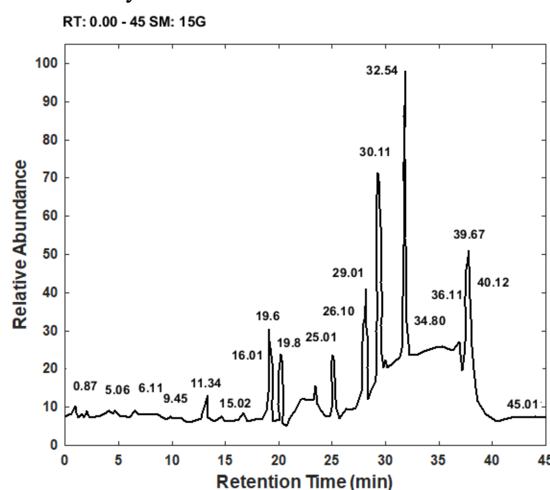

Figure 3: HR-LCMS chromatogram

3.4. Estimation of anti-oxidant activity

By utilizing four well-known assay methods, namely ABTS, DPPH, FRAP, and H_2O_2, the in vitro anti-oxidant activity was determined.

3.4.1. ABTS assay

At different concentrations, the RS potential of the ME of *K.nemoralis* was determined in the ABTS assay. It also

possessed a concentration-reliant PI activity on ABTS radical. The plants' inhibition of ABTS RS activity with standard AA and ME is given in Table 7.

Table 7: % of Inhibition of AA and the ME of *K.nemoralis* using ABTS assay

S. No	Concentration (µg/ml)	% Inhibition	
		AA	*% inhibition (extract)*
1	10	31.25	15.50
2	20	43.15	23.17
3	40	57.28	27.60
4	60	76.15	33.73
5	80	82.66	49.40
6	100	97.10	53.32
IC_{50}		34.67	175.57

As per the result, the PI for AA ranged from 31.25 to 97.10% and *K.nemoralis* extract ranged from 15.50 to 53.32 %. This demonstrated that the PI of the plant material improved with the upsurge in the ME's concentration. The IC_{50} values for standard AA and *K.nemoralis* were 34.67 and 175.57, respectively. In Figure 4, the graphical illustration of the PI of AA and the plant material using the ABTS assay is illustrated.

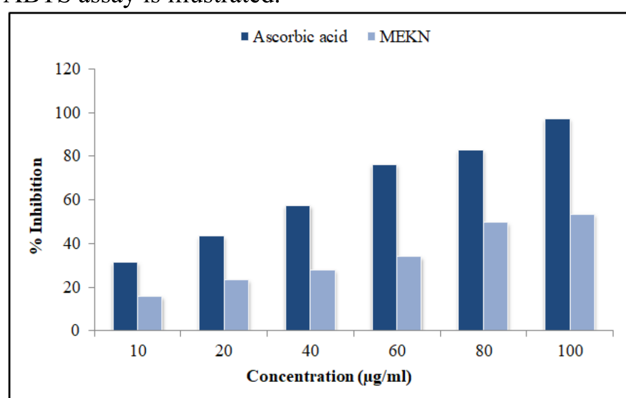

Figure 4: % of Inhibition using ABTS assay

3.4.2. DPPH assay

The DPPH assay method was used to screen for anti-oxidants due to its efficiency and capacity to screen many samples at one time, and its inherent capability to target bioactive compounds, even at low concentrations. At various concentrations, the AA's DPPH RS potential and the plant's ME were determined. In Table 8, the PI of AA and ME for selected plants utilizing DPPH is depicted.

Table 8: Percentage of Inhibition of AA and ME of *K.nemoralis* using DPPH assay

S. No	Concentration (µg/ml)	% Inhibition	
		AA	***K.nemoralis* extract**
1	10	42.85	16.61
2	20	48.72	28.35
3	40	55.49	29.63
4	60	57.83	35.93
5	80	68.82	36.36
6	100	79.77	37.22
IC_{50}		29.34	150.78

The PI against various concentrations of extract of *K.nemoralis* and AA was utilized for computing the IC_{50} (µg/ml). The IC_{50} value was determined as 29.34 in AA and 150.78 in the ME of *K.nemoralis*. The PI values are elevated with an upsurge in plant extract in the assay mixture. In Figure 5, the graphical illustration of the PI of AA and the plant material using the ABTS assay is demonstrated.

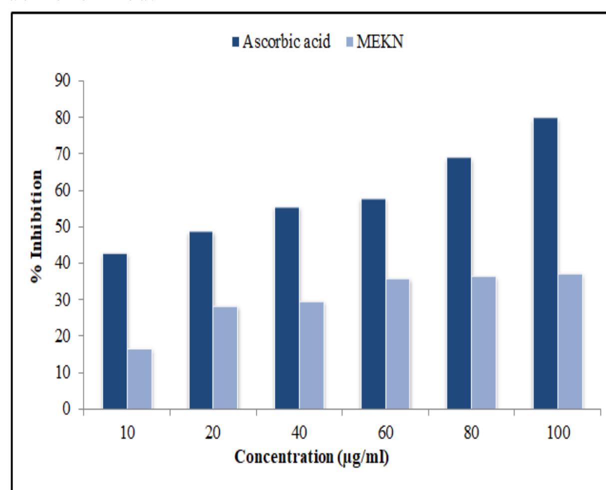

Figure 5: % of Inhibition using DPPH assay

3.4.3. FRAP assay

In Table 9, the FRAP test outcomes for the ME of *K.nemoralis,* in contrast with the ordinary AA at 700 nm, are exhibited.

Table 9: Percentage of Inhibition of AA and ME of *K.nemoralis* using FRAP

S. No	Concentration (µg/ml)	% Inhibition	
		AA	***K.nemoralis* extract**
1	10	41.56	13.53
2	20	54.08	21.56
3	40	56.72	28.43
4	60	68.46	38.66
5	80	75.55	44.46
6	100	87.56	52.57
IC_{50}		21.81	121.65

The PI at different concentrations, for AA and the ME of *K.nemoralis* were 41.56, 54.08, 56.72, 68.46, 75.55, 87.56, and 21.81, and 13.53, 21.56, 28.43, 38.66, 44.46, and 52.57, respectively. The PI of the plant material increased with the upsurge in the extract's concentration, exhibiting the plant material's higher anti-oxidant activity. The IC50 values of the standard and plant material were 21.81 and 121.65, respectively. In Figure 6, the graphical illustration of the PI of AA and the plant material using the FRAP assay is depicted.

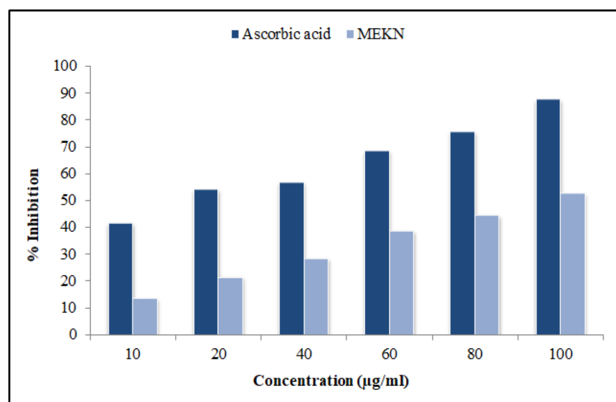

Figure 6: % of Inhibition using FRAP assay

3.4.4. H_2O_2 assay

Table 10 presents the results of an H_2O_2 assay by comparing the AA's anti-oxidant activity with that of ME of *K.nemoralis*.

Table 10: H_2O_2 scavenging – % inhibition by AA and K. nemoralis extract

S. No	Concentration (µg/ml)	% Inhibition	
		AA	*K.nemoralis extract*
1	10	42.56	7.46
2	20	50.34	15.67
3	40	56.65	23.95
4	60	63.47	35.48
5	80	78.56	39.20
6	100	82.59	48.57
IC_{50}		29.37	100.67

The PI for both AA and *K.nemoralis* extract was augmented with increasing concentration. When compared to the *K.nemoralis* extract, the AA showed a higher PI at all tested concentrations. The IC_{50} value was significantly lower for AA (29.37) than the *K.nemoralis* extract (100.67 µ), indicating that AA was a more potent anti-oxidant in this assay. Nevertheless, the anti-oxidant potential was lower contrasted to other RS assays. In Figure 7, the graphical illustration of the PI of AA and the plant material using the H_2O_2 assay is depicted.

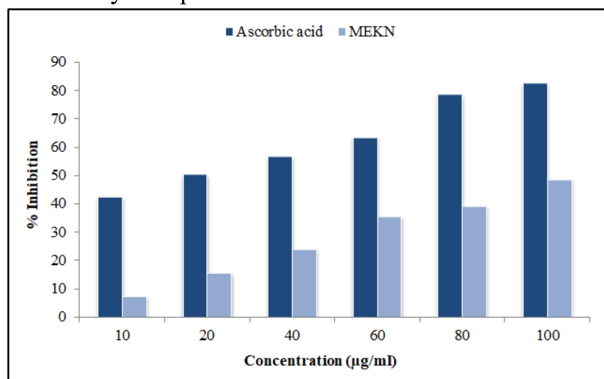

Figure 7: % of Inhibition using H_2O_2 assay

3.5. In vitro anti-diabetic activity

The in vitro antidiabetic characteristics of *K.nemoralis* extract were evaluated by determining its inhibitory effect on important carbohydrate metabolism enzymes (α-amylase and α-glucosidase), the two enzymes that directly influence postprandial BG levels. The results obtained through the α-amylase inhibition assay are summarized in Table 11.

Table 11: In vitro antidiabetic activity of α-amylase method of MEKN

S. No	Concentration (µg/ml)	% Inhibition	
		Acarbose	*K.nemoralis*
1	10	30.43	28.34
2	20	34.75	37.24
3	40	49.22	43.56
4	60	57.58	58.71
5	80	63.91	69.14
6	100	74.67	82.42
IC_{50}		50.25	53.47

A progressive, concentration-dependent enhancement in α-amylase inhibition was observed, indicating that the enzyme's activity decreased proportionally with increasing extract concentration. The PI of Acarbose and *K.nemoralis* with concentrations like 10, 20, 40, 60, 80, and 100µg/ml were 30.43, 34.75, 49.22, 57.58, 63.91, and 74.67, and 28.34, 37.24, 43.56, 58.71, 69.14, and 82.42, respectively. The extract and the acarbose's IC_{50} value were found to be 53.47 and 50.25, respectively. Figure 8 depicts the illustration of the α-amylase assay's PI.

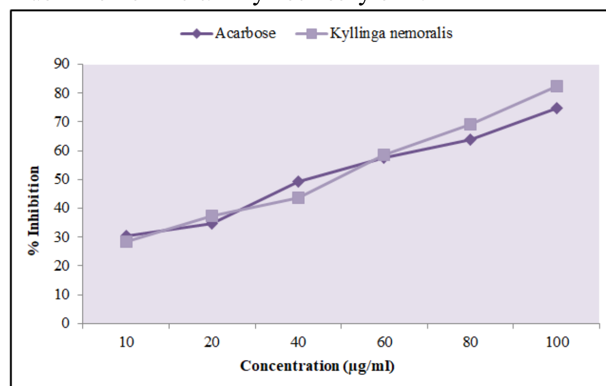

Figure 8: Percentage inhibition of α-amylase activity

The anti-diabetic activity results using the α-glucosidase method are presented in Table 12.

Table 12: α-Glucosidase Inhibition Activity (% Inhibition) of Acarbose and *K.nemoralis*

S. No	Concentration (µg/ml)	Acarbose	*K.nemoralis*
1	10	39.67	24.35
2	20	56.08	33.98
3	40	64.01	41.67
4	60	72.56	56.45
5	80	85.71	67.35
6	100	92.27	78.46
IC_{50}		50.72	51.52

A considerable inhibitory action on α-glucosidase enzyme was shown by the ME of *K.nemoralis.* A concentration-reliant augmentation in PI was shown by the PI at 10-100 µg/ml concentrations of the plant extract. The PI changes from 39.67 to 92.27 for acarbose and from 24.35 to 78.46 for the plant extract. The graphical representation of the PI of the α-glucosidase assay is portrayed in Figure 9.

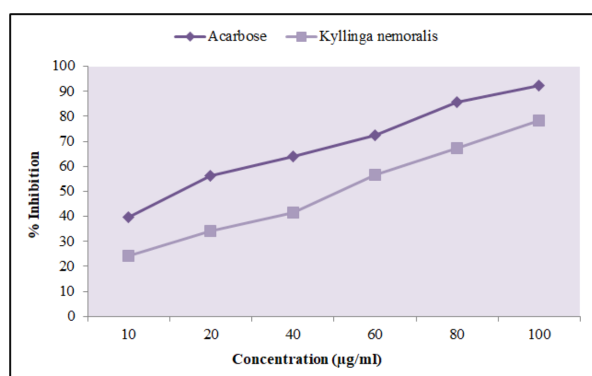

Figure 9: PI using α-glucosidase assay

3.6. In vivo anti-diabetic activity

Rats were orally treated with a single dose of numerous concentrations of *K.nemoralis*, like 500, 1000, 1700, 1800, and 2000 mg/kg, to carry out acute toxicity. The BW and BG levels were recorded for the control and treated groups at 1, 4, 7, 11, and 15 days. In Table 13, the results of BW among the normal control, disease control, and the treated groups are represented.

Table 13: Comparative Body Weight (g) of Experimental Groups During the Study

Group	Day 1	Day 4	Day 7	Day 11	Day 15
Normal Control (NC)	217.22 ± 4.14	217.94 ± 4.52	218.86 ± 5.18	219.61 ± 5.49	220.54 ± 5.91
Disease Control (DC)	222.82 ± 5.70	226.37 ± 5.96	229.61 ± 6.08	233.08 ± 6.09	235.46 ± 5.93
Treated with *K.nemoralis*	208.08 ± 6.58	209.96 ± 7.06	211.83 ± 8.23	213.52 ± 8.81	216.04 ± 9.39

After the treatment, when analogized with the control, BW was reduced. The rats' BW increased as the treatment days increased. No statistically significant differences were found betwixt the normal control group and the treated group. In addition, no mortality signs were found. In Table 14, the results of BG levels among the normal control, disease control, and the treated groups are portrayed.

Table 14: Blood Glucose Profile of Experimental Rats

Day	NC (mg/dL)	DC (mg/dL)	Treated (mg/dL)
1	115.54 ± 1.56	203.09 ± 1.71	186.24 ± 2.03
4	116.34 ± 1.74	205.54 ± 2.02	176.31 ± 1.83
7	117.50 ± 1.48	206.72 ± 1.67	142.57 ± 2.06
11	118.46 ± 1.92	208.36 ± 2.11	126.92 ± 1.63
15	119.57 ± 1.12	209.01 ± 1.58	118.67 ± 1.31

In Table 14, the impact of extract treatment on BG levels among the treated and control groups was shown. The diabetic rats treated with *K.nemoralis* of standard group 3 exhibited considerable reductions in BG on day 1, 4, 7, 11, and 15 when analogized with the diabetic control group 2. It is detected that the *K.nemoralis* treatment mitigated the diabetic rats' BG levels successfully towards the normal level within 15 days.

Histopathology images with interpretation

As per the histological study, the *K.nemoralis* had a regenerative effect on pancreatic tissue. The pancreas of disease control showed significant changes, including shrinking in size, altered architecture, and reduced numbers. The pictorial representation of the pancreas from the rats of the normal control, disease control, and the treated group is given in Figure 10.

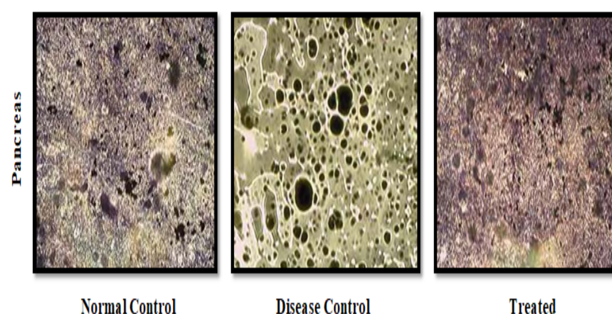

Figure 10: Histological study of the pancreatic tissues of control and treated rats

4. DISCUSSION

Analyzing the anti-diabetic activity of the plant named Kyllinga nemoralis was the aim of this study. HR-LCMS analysis was deployed in the study to detect bioactive compounds present in the ME of Kyllinga nemoralis. The investigation conducted by (Chithra & Nair, 2021) documented the presence of Carbohydrates, Phenols, Flavonoids, Flavones, Tannins, Saponins, Steroids, Terpenoids, Coumarins, Quinones, Lignin, and Fixed oils and fats. Meanwhile, (Wahab & Rahman, 2023) noted that *K.nemoralis* ME did not contain saponins. The examination indicated that the *K.nemoralis* ME had TPC and TFC of 92.26 0.16 mg GAE/g and 19.87 0.16 mg QE/g, respectively. (Datta et al., 2022) found that *K.nemoralis* with CH_3OH had TPC of 122.56±1.28 mg GAE/g and TFC of 20.66±0.55 mg Rutin equivalent/g. For another plant named *Glycyrrhiza glabra* with aqueous and alcoholic extracts, the TPC and TFC were 23.26 and 18.70 µg GAE/mg and 6.62 and 15.03 µg QE/mg, correspondingly (Gupta et al., 2016). The HR-LCMS analysis of the plant found a total of 19 bioactive compounds, such as 3-(Prop-2-enoyloxy)tetradecane, 1-chloro-1-fluoroethane, 2-butanone, 3-pentanone, 7-methylbicyclo[3.2.0]hept-3-ene-2-one, 2-methyl butanoic acid methyl, 1,3-dichlorobenzene, Cycloheptasiloxane, tetradcamethyl, p-Hydroxybenzoicacid, Diethyl Pthalate, Cyclooctasiloxane, hexadecamethyl, Nonane 5- (2-methyl propyl), Heptasiloxane, hexadecamethyl, 1,2 Benzene dicarboxylic acid, bis (2 methyl propyl), Tetradecane 2,6,10 trimethyl, 13,16 Octadecadienoic acid, methyl ester, Cyclo 3octa siloxane hexadecamethyl, Cyclononasiloxane octadecamethyl, and Tetracosamethyl cyclododeca siloxane. Likewise, (Bhavani et al., 2025) identified 18 bioactive compounds from the *K.nemoralis's* ME. (Datta et

al., 2022) recognized 9 bioactive compounds from the *K.nemoralis's* dried plant material.
A concentration-dependent inhibition activity in the assay was exhibited by the in vitro anti-oxidant activities of the ME of *K.nemoralis*. Next, PI values augmented with the upsurge in concentration of the ME of *K.nemoralis* in the assay mixture, such as DPPH, ABTS, FRAP, and $H_2 0_2$. Similarly, (Gupta et al., 2016) indicated that the PI augmented with the upsurge in concentration for DPPH, ABTS, FRAP, and $H_2 0_2$ for another plant, *Glycyrrhiza glabra.* According to (Rajagopal et al., 2016), the free RS activity of the *K.nemoralis* extract was indicated by the significant inhibitory activity in the in vitro anti-oxidant studies of the extract with reduction of ferric ions. The study found a dose-dependent upsurge in percentage inhibitory activity against α-amylase and α-glucosidase enzymes. The PI of α-amylase and α-glucosidase assay with a concentration of 100µg/ml was found to be 82.42% and 78.46% for the ME of *K.nemoralis,* respectively. (Mir et al., 2015) stated that for the ME, the PI of α-amylase was found to be 84.23%. Similarly, the PI of α-glucosidase was 79.93% for the ME. According to the in-vivo diabetic study, the ME of *K.nemoralis* treatment mitigated the BG levels and enhanced the BW in the diabetic rats towards a normal level in 15 days. The histopathological analysis of albino Wistar rats indicated that the *K.nemoralis* had a regenerative impact on pancreatic tissue. In a separate study, the author (Elshamy et al., 2017) examined another plant *named Cyperus laevigatus* and demonstrated that the diabetic rats' pancreas treated with the plant extract exhibited dramatic suppression of all abnormal histological modifications than the diabetic group.

5. CONCLUSION

In the current study, the phytochemical compounds available in the *K.nemoralis* were investigated utilizing HR-LCMS analysis. By utilizing in vitro and in vivo approaches, the diabetic activity of the plant was examined in this study. The existence of carbohydrates, phenols, flavonoids, tannins, saponins, alkaloids, steroids, terpenoids, and quinones was exposed by the qualitative phytochemical screening. As per the HR-LCMS analysis, 19 bioactive compounds with a retention time of 0 to 45 minutes were found. The robust activity in the plant was shown by the anti-oxidant assays. Higher scavenging efficiency in the ME of *K.nemoralis* (IC_{50}:175.57 µg/mL) was demonstrated by the ABTS assay. The ME of *K.nemoralis* had the potential to manage BG by inhibiting enzymes like α-amylase and α-glucosidase. As per the histological study, *K.nemoralis* had a regenerative effect on pancreatic tissue. Nevertheless, only one plant was used in this study for the estimation of phytochemicals using the HR-LCMS methodology. In addition, the study didn't compare the anti-diabetic potential of *K.nemoralis* with other plants belonging to the family Cyperaceae. Hence, this limitation will be addressed in future studies, which will utilize two or more other plants for the analysis

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