Utility of Raman spectroscopy in diabetes detection based on biomarker Raman bands and in antidiabetic efficacy studies of herbal extract *Rotheca myricoides Hochst*

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Abstract
Diabetes is a disease characterized by hyperglycaemia because of insufficient or nonproduction of insulin from the pancreas. Establishing prediabetic and diabetic condition often involves monitoring levels of glucose and some amino acids in blood using nonrapid and label-dependent methods. This work reports on a method with a potential of being used for quick label-free detection of diabetes mellitus type II based on Raman spectroscopy of blood applied onto a conductive silver-smeared glass slide. We show that Raman spectral profile from blood of streptozotocin-induced diabetic Sprague Dawley rats emanates from overlap of signals from valine, leucine, isoleucine, creatine, glucose, and fructose. The Raman spectral bands associated with these biomolecules have the potential of being used in prediabetic detection and diabetes prediction. Characteristic intense peaks in diabetic rat’s blood spectra were centred at wave numbers 537 cm⁻¹ associated with valine’s CO₂⁻ rocking vibration, 829 cm⁻¹ assigned to CH₂ rocking vibration in leucine and 917–960 cm⁻¹ ascribed to C–C and C–N stretching and CH₃ rocking vibrations in various biomolecules. The average intensities of these bands were sensitive to antidiabetic drug administration on the rats as their values approached those of nondiabetic rats and so could be used as diabetes biomarker bands. Statistical analyses together with evaluation of average intensities of these biomarker bands showed that the herbal extract *Rotheca myricoides Hochst* had greater antidiabetic effect at low dose (50 mg/kg of body weight) than at high dose (100 mg/kg of body weight). A similar result was seen with area under curve values and could act as an additional parameter in diabetes detection and prediction.

KEYWORDS
Raman spectroscopy, type II diabetes, biomarker Raman bands, *Rotheca myricoides Hochst*

1 | INTRODUCTION

Globally, by the end of year 2018, it was estimated that more than 500 million people will be living with diabetes mellitus type II.[¹] The prevalence of this condition, which is associated with elevated blood glucose levels, is rising in middle- and low-income countries. In diabetic patients, production of insulin, the hormone that regulates blood sugar levels in the body, is either low or nonexistent.[²] When glucose is elevated in blood as in...
diabetic patients, it can lead to serious long-term health complications including hypertension, stroke, blindness, and limb amputation.\cite{3,4}

Diabetes is an irreversible metabolic condition and is only managed using proper medication and diet. A prediabetic condition is manifested through impaired fasting glucose and elevated levels of circulating branched-chain amino acids (BCAA) in blood.\cite{5-10} At this stage (i.e., prediabetic), appropriate interventions that include physical exercise, low or no sugar diet, among others, can prevent development of type II diabetes mellitus.\cite{11} Measurement of blood sugar and BCAA (leucine, isoleucine, and valine) involves using a glucometer\cite{12,13} and ion-exchange high performance liquid chromatography,\cite{5,14} respectively. These techniques involve use of labels, are time consuming, and costly. In this work, we show that Raman spectroscopy can be an alternative technique for performing quick and label-free detection of diabetes (or prediabetes) in blood with Raman spectral bands associated collectively with valine, leucine, isoleucine, creatine (creatine monohydrate), and sugar (glucose and fructose) as biomarkers. In Birech et al.\cite{12} it was shown that the intensity of Raman bands associated with isoleucine and leucine is different between blood from diabetic, treated, and nondiabetic rats.

Raman spectroscopy involves shining a laser light onto the sample of interest and the wavelength-shifted (Raman scattered) radiation collected, dispersed in a spectrometer and recorded. This radiation emanates from radiative vibrational relaxations in excited molecules in the sample and thus the resultant spectral profile is unique to them. This spectroscopic technique is increasingly generating a lot of interest in diabetes detection with spectral bands associated with some biomolecules acting as biomarkers. Some of the biomolecules that have been demonstrated using Raman spectroscopy to have a great potential in the detection and monitoring of diabetes mellitus type II include blood glucose,\cite{10,15-18} haemoglobin,\cite{19} lipids in erythrocyte membranes,\cite{20} and branched chain amino acids.\cite{12} Its use (i.e., Raman spectroscopy) in comparative efficacy studies of antidiabetic medications, both conventional and traditional, has been reported elsewhere.\cite{12}

Here, we report on Raman spectroscopic studies done on blood extracted from diabetic and nondiabetic Sprague Dawley (SD) rats. The blood was pipetted onto a conductive silver paste smeared glass slide and excited using a 785-nm laser.\cite{12,21} Induction of diabetic conditions in the rats was achieved through injection of a beta cell (ß-cell) toxin called streptozotocin (STZ).\cite{22-24} ß-cells situated in the pancreases are known to make insulin and their degeneration results in type II diabetes mellitus.\cite{23} Raman spectral profiles from SD rats treated with the commercially available conventional antidiabetic drug pioglitazone\cite{2} and those treated with a traditional antidiabetic herbal extract Rotheca myricoides Hochst were obtained and compared. The herb, Rotheca myricoides Hochst., formerly known as Clerodendrum myricoides,\cite{25} belongs to the genus Rotheca, the largest genus of the family Verbenaceae\cite{26} and is used traditionally in diabetes management.\cite{27} Most species of this plant are found in Africa (Kenya and Uganda), some in Southern Asia, and a few in America and Australia.\cite{28} Results obtained showed that Raman spectral profiles from blood of diabetic rats differed from those from nondiabetic with characteristic bands associated with BCAA (leucine, isoleucine, and valine), creatine (creatine monohydrate), glucose, and fructose being prominent in the former samples. The intensities of these bands were sensitive to antidiabetic drug administration on the rats, and their values were observed to decrease to a value close to those of nondiabetic rats and so could be used as diabetes biomarker bands. The area under curve (AUC) values of Raman spectral profiles from diabetic, treated, and nondiabetic rats are here shown to be potential parameter in diabetes detection and prediction.

2 | MATERIALS AND METHODS

2.1 | The animals (Sprague Dawley rats)

Fifty (25 males and 25 females) four-week-old Sprague Dawley (SD) rats weighing 60–100 g obtained from the Department of Zoology, University of Nairobi, were used in the study. These animals were then grouped into five of 10 members each as follows: Diabetic, D (10 rats; fed on high fat and high sugar diet), low dose, Ld (10 rats; fed on high fat and high sugar diet, administered extract Rotheca myricoides Hochst at low dose), high dose, Hd (10 rats; fed on high fat and high sugar diet, administered extract Rotheca myricoides Hochst at high dose), Pioglitazone, Pg (10 rats; fed on high fat and high sugar diet, administered pioglitazone), nondiabetic, Nd (10 rats, fed on normal diet). The animals were housed in metallic cages of dimensions 109 cm by 69 cm by 77.5 cm at the Department of Medical Physiology, University of Nairobi. The floors of these cages were covered with wood shavings that were constantly replaced trice per week during cleaning. After habituation for 8 days, they were fasted for 6–8 hr, anaesthetized by application of lidocaine and 10 min wait before 100 μL of blood is drawn via lateral tail vein sampling. Nondiabetic status would then be confirmed through blood glucose level measurement using a commercially available glucometer (StatStrip Xpress Nova Biomedical, Waltham MA, USA). During the study
period (16 weeks), apart from the nondiabetic group, all the rats were fed on high fat and high sugar diet. The fat diet was prepared by adding 45 g of vegetable cooking fat (Frymate, Pwani industries) to 225 g of standard chow pellets (Unga Feeds ltd, Nairobi). The high sugar diet on the other hand consisted of 20 g of sugar (99 % fructose) dissolved in 100 ml of water and was fed ad libitum throughout. The nondiabetic group (10 rats) was fed on a normal diet (standard chow pellets with the following nutritive value: carbohydrate 70%, protein 20%, fat 10%).

On the 42nd day after giving the above diet, the rats were injected intraperitoneally with a low dose of streptozotocin (30 mg/kg) to induce type II diabetes because all the target groups (diabetic, low dose, high dose, and pioglitazone) had fasting blood glucose levels that were lower than the diabetic threshold of 7.0 mmol/L. Fasting blood glucose and oral glucose tolerance test was conducted every week, and rats with glucose levels >7.0 mmol/L and with impaired oral glucose tolerance (≥11.1 mol/L) were considered diabetic. Administration of the herbal extract and pioglitazone to the rats was via oral gavage with the following dosage: diabetic (distilled water), low dose of herbal extract Rotheca myricoides Hochst (50 mg/kg of body weight), high dose of herbal extract Rotheca myricoides Hochst (100 mg/kg of body weight), and pioglitazone (20 mg/kg of body weight).

2.2 | The herbal extract of Rotheca myricoides (Hochst)

The identity of fresh whole herbal plant Rotheca myricoides (Hochst) collected from their natural habitat (Machakos county, Kenya) was first verified at University of Nairobi herbarium and a voucher specimen deposited therein. The harvested plant samples were air dried for a week before being ground into a dry powder. The powder was macerated in distilled water for 25 min in a weight/volume ratio of 1:10. The solution was then filtered in two stages. Coarse filtration done using cotton wool, and fine filtration of the resulting filtrate done through filter papers. The filtrate was then lyophilized (Christ Alpha 1-6 Medizinischer Apparatus Osterode/Harz, Germany) to obtain the freeze-dried extract. The extract was weighed, placed in amber coloured sample bottles, and stored in the deep freezer environment. For plotting, ORIGIN (Originpro 9.1) software was used.

2.3 | Raman spectroscopy

Raman spectroscopy was carried out using confocal Raman system (STR, Seki Technotron Corp) equipped with a 785-nm laser and a spectrometer (Princeton Instruments). Silver conductive paint (SPI supplies, SPI#04961-AB) with about 43% silver nanoparticles were smeared onto a microscope glass slide (see Figure S1) to act as Raman sample substrates as described in Birech et al.[12] Calibration of the device was also done in a similar manner as in Birech et al.[12] The experimental parameters for this study were as follows: grating, 600 groves/mm; centre wavelength, 850.97 nm (980 cm⁻¹); excitation power at sample position, 9 mW; spot size at sample position, 68 μm; exposure time, 10 s; spectral accumulation, 5 s; X10 microscope objective (Olympus MP2000FN, 0.3NA). A small amount of blood (~10 μL, whole blood) was pipetted onto the silver smeared glass slide and air dried for 1 hr. For samples from each rat in the five groups (i.e., nondiabetic, diabetic, low-dose herb, high dose herb, and pioglitazone treated), six spectra were collected at different random spots and recorded making 60 per group. Hence, 300 Raman spectral data were collected in the study.

2.4 | Data processing

Data preprocessing involved autofluorescence background subtraction and smoothing using Vancouver Raman algorithm developed by Zhao et al[29] with the following settings: Boxcar smooth size, 5; polynomial fit, fifth order; spectral data range, 350–1,800 cm⁻¹. It was then followed by a series of normalizations: to maximum intensity, to AUC and subtraction of minimum value per data set in that order in MATLAB 2017a scripting environment. For plotting, ORIGIN (Originpro 9.1) software was used.

2.5 | Spectral similarity

To compute the spectral similarity between spectral data from blood of diabetic rats (D) and those from a sum of individual average spectra from creatine monohydrate, valine, leucine, isoleucine, glucose, and fructose solids (Solid, S; i.e., Raman spectra of creatine + valine + leucine + isoleucine + glucose + fructose; black line, solid), cosine similarity metric[30–32] was used. Cosine similarity is defined as

$$\cos \theta = \frac{D \cdot S}{|D||S|} = \frac{\sum_i D_i S_i}{\left(\sum_i D_i^2\right)^{\frac{1}{2}} \left(\sum_i S_i^2\right)^{\frac{1}{2}}}$$

Raman intensity at wave number i of spectra from blood...
of diabetic rats and from the combined solid spectra respectively. Similar or identical (dissimilar) spectral data sets will give a value of 1 (0) whereas opposite sets will give a value of -1. A large positive value of cosθ implies that the spectra are identical.[31,32]

2.6 | Principal component analysis

Principal component analysis (PCA) is an analytical technique that utilizes spectral patterns in segregating between data sets. Variations are expressed in terms of percentage variance. A linear combination of wave numbers followed by ranking is then done. The resulting multivariate data set is represented on a set of perpendicular axes referred to as principal components (PCs). The PC with the highest variance is called PC1, followed by PC2 and so on.[33–35] Each of the spectral data set is displayed as a point (score) on a PC plane.[21] In this work, PCA was applied on a combined spectral data set from the five different rat groups, that is, nondiabetic, diabetic, *Rotheca myricoides* (Hochst) treated at low and high dose, and pioglitazone treated. Score plots of the first three PCs with the highest variance were then done.

2.7 | Statistical analyses

To identify which Raman bands provide a greater potentiality for use as diagnostic biomarker bands, analysis of variance (ANOVA) technique was used. The bands showing higher variance across the spectral data groups is then chosen as significant. In our ANOVA analysis, the hypothesis assessed was that there were no variations in the means of the spectral data groups, that is, the means were the same. A plot of variance between the spectral groups versus the wave numbers was then done to find the significantly important bands. An alpha value of .05 was used. To compare the means or variance in a pair of spectral data, that is, between diabetic versus nondiabetic and treated versus nondiabetic, a two-sample Student t test was used.

2.8 | Ethical approval

Ethical approval for the study was granted by the Biosafety, Animal Care and Use Committee, Faculty of Veterinary Physiology, University of Nairobi.

3 | RESULTS AND DISCUSSION

3.1 | Diabetic, treated, and nondiabetic rats

Spectral profiles from blood of streptozotocin-induced diabetic rats (D, red line) differed from those of nondiabetic rats (ND, black line) as seen in Figure 1a. Also observed was that Raman spectra from blood of rats administered antidiabetic herbal extracts (low dose, LD, and high dose, HD) and the conventional antidiabetic drug pioglitazone (Pio) displayed profiles that were like those from nondiabetic rats. Prominent characteristic Raman bands from diabetic rats were seen at around 537, 775, 829, 918–960, and 1,051 cm⁻¹, whereas for the treated and nondiabetic rats were at around 476, 635, 1,210, and 1,572 cm⁻¹ (see Figure S3 and Table 1 for tentative vibrational assignments).
The Raman spectral profile from blood of diabetic rats could be reproduced by summing the individual average spectra from creatine monohydrate, valine, leucine, isoleucine, glucose, and fructose solids (i.e., Raman spectra creatine + valine + leucine + isoleucine + glucose + fructose; black line, solid) as displayed in Figure 1b (see also Figure S2). A cosine similarity value of 0.91 was obtained between the two spectral data sets showing that they were identical. This implied that the levels of the respective biomolecule are significantly changed in streptozotocin-induced diabetic rats. Indeed, a number of studies have shown that levels of valine, leucine, isoleucine, and creatine, and fructose are elevated in blood of diabetic patients. Fructose levels in blood too get elevated vibration in leucine and 917 cm\(^{-1}\) and 960 cm\(^{-1}\) associated to leucine, 537 cm\(^{-1}\) (ascribed to leucine) 635 cm\(^{-1}\) ascribed to fructose, 829 cm\(^{-1}\) assigned to CH\(_2\) rocking vibration in leucine and 1,050 cm\(^{-1}\) ascribed to C–C and C–N stretching vibrations [36] in various biomolecules (see Figures 1 and S3). These bands together with those at 1,051, 1,210, 1,396, and 1,572 cm\(^{-1}\) (see Table 1 for their assignments) could be used as reference biomarker bands as they displayed significant mean variation (see Figure S3) across the groups (i.e., diabetic, treated, and nondiabetic). The diagnostic significance of Raman bands centred at 537, 829, 937, and 1,050 cm\(^{-1}\) are displayed in Figure 2 where their respective average intensities were observed to decrease in value upon administration of antidiabetic treatments (pioglitazone, PG, and *Rotheca myricoides* Hochst) extracts at low dose, LD, and high dose, HD) to the rats. The Raman spectral intensity decrease of treated rats (LD, HD, and PG) approached those of nondiabetic (ND) rats. To evaluate the similarities between these spectra based on their mean values, Student t test analysis was done and the obtained results are displayed in Tables 2 and 3. Based on the obtained p values and the means (see Tables 2 and 3, respectively), in terms of similarity with the nondiabetic (ND) spectra, it was found that LD, PG, HD, and D were more similar in decreasing order. This implied that the herbal extract *Rotheca myricoides Hochst* was more effective at low dose (50 mg/kg) than both using the commercially available antidiabetic drug pioglitazone (PG) and the herbal extract at high dose (100 mg/kg). The Raman band intensity analysis displayed in Figure 2 also confirms the argument that the extract at low dose was more effective. Besides, the results prove that the locally used traditional antidiabetic herbal extract was equally effective against type II diabetes mellitus (especially at low dose) as the commercially available antidiabetic drug pioglitazone. The selected Raman bands are potential type II diabetes mellitus biomarkers bands. The peaks at 476 cm\(^{-1}\) (ascribed to leucine) 635 cm\(^{-1}\), 1,210 cm\(^{-1}\) (associated

<table>
<thead>
<tr>
<th>Wave number (cm(^{-1}))</th>
<th>Diabetic</th>
<th>Treated (HD, LD, and PG) and nondiabetic (ND)</th>
<th>Component</th>
<th>Tentative vibrational assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>476</td>
<td>-</td>
<td>√</td>
<td>Leucine</td>
<td></td>
</tr>
<tr>
<td>537</td>
<td>√</td>
<td>-</td>
<td>Valine</td>
<td>CO(_2^-) rocking vibration in valine [36]</td>
</tr>
<tr>
<td>635</td>
<td>-</td>
<td>√</td>
<td>Fructose</td>
<td></td>
</tr>
<tr>
<td>775</td>
<td>√</td>
<td>-</td>
<td>Valine; leucine; isoleucine; glucose</td>
<td>CO(_2^-) bending vibration in valine [36] and in leucine [37]; C–CO stretching vibrations in leucine [36]</td>
</tr>
<tr>
<td>829</td>
<td>√</td>
<td>-</td>
<td>Uric acid; leucine</td>
<td>CH(_2) rocking in leucine [38]</td>
</tr>
<tr>
<td>918</td>
<td>√</td>
<td>-</td>
<td>Glucose</td>
<td>C–C stretching vibrations [36,37]</td>
</tr>
<tr>
<td>945</td>
<td>√</td>
<td>-</td>
<td>Creatine MH</td>
<td>C–C and C–N stretching vibrations [36,37]</td>
</tr>
<tr>
<td>1,051</td>
<td>√</td>
<td>-</td>
<td>Leucine; creatine MH</td>
<td></td>
</tr>
<tr>
<td>1,210</td>
<td>-</td>
<td>√</td>
<td>Glucose and fructose</td>
<td></td>
</tr>
<tr>
<td>1,396</td>
<td>√</td>
<td>-</td>
<td>Fructose</td>
<td></td>
</tr>
<tr>
<td>1,572</td>
<td>-</td>
<td>√</td>
<td>Silver paste; uric acid</td>
<td>Stretching vibrations of CO(_2^-) [36]</td>
</tr>
</tbody>
</table>

Note. The symbols “√” and “−” in the table mean the peak is “present” and “absent,” respectively.
with glucose and fructose), and 1,572 cm\(^{-1}\) (due to uric acid; see Table 1) that were unique in spectra of treated and nondiabetic rats could also be used as treatment efficacy examination bands.

Given that the Raman spectral profiles from diabetic and the treated rats including those from normal were distinctly different, their AUC values were also expected to be different. If that is correct, then the AUC values could be an additional diabetes type II detection parameter during screening. Figure 3 shows that indeed the AUC values of Raman spectra from diabetic, treated, and nondiabetic rats were different and could be used both in distinguishing between diabetic and nondiabetic rats and in assessing the antidiabetic efficacy of herbal extracts in comparison with commercially available conventional antidiabetic medications. Specifically, the obtained AUC values from diabetic, nondiabetic, treated (HD, LD, and PG) were 101, 189, 202, 190, and 192 square units, respectively. The AUC value of 190 square units obtained from rats administered low dose (LD) concentrations of Rotheca myricoides Hochst extract was closest to 189, the value from spectra of healthy/nondiabetic rats and confirming the results that the effective dose of the extract was the low dose, LD (i.e., 50 mg/kg of body weight). The \(p\) value (or \(t\) score) of .6130 obtained in the Student \(t\) test for LD versus ND spectra comparison (see Table 2) which was greater than the chosen alpha value of .05 also confirmed that the two were 61% similar and showing the efficacy of

### TABLE 2

The results from a two value Student \(t\) test between the various Raman spectral data groups

<table>
<thead>
<tr>
<th>Group</th>
<th>(p) value</th>
<th>(\alpha) value</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD versus LD</td>
<td>.2268</td>
<td>&gt;.05</td>
<td>Similar</td>
</tr>
<tr>
<td>HD versus PG</td>
<td>.0085</td>
<td>&lt;.05</td>
<td>Not similar</td>
</tr>
<tr>
<td>HD versus ND</td>
<td>.0852</td>
<td>&gt;.05</td>
<td>Similar</td>
</tr>
<tr>
<td>LD versus PG</td>
<td>.1534</td>
<td>&gt;.05</td>
<td>Similar</td>
</tr>
<tr>
<td>LD versus ND</td>
<td>.6130</td>
<td>&gt;.05</td>
<td>Similar</td>
</tr>
<tr>
<td>D versus ND</td>
<td>(1.025 \times 10^{-17})</td>
<td>&lt;.05</td>
<td>Not similar</td>
</tr>
<tr>
<td>PG versus ND</td>
<td>.3519</td>
<td>&gt;.05</td>
<td>Similar</td>
</tr>
</tbody>
</table>

*Note*. The \(p\) values together with comparison to the set \(\alpha\) value of 0.05 is also shown. Spectral data pairs with \(p\) values greater than the \(\alpha\) value were similar otherwise they are dissimilar.

### TABLE 3

The means and variance of the obtained Raman spectral data sets from diabetic (D), nondiabetic (ND), and the treated rats (LD, HD, and PG)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>Variance ((\times 10^{-6}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>0.00199</td>
<td>0.92</td>
</tr>
<tr>
<td>HD</td>
<td>0.00149</td>
<td>1.03</td>
</tr>
<tr>
<td>LD</td>
<td>0.00155</td>
<td>1.05</td>
</tr>
<tr>
<td>PG</td>
<td>0.00162</td>
<td>1.07</td>
</tr>
<tr>
<td>ND</td>
<td>0.00158</td>
<td>1.03</td>
</tr>
</tbody>
</table>

*Note*. The variance of the spectral data from the treated rats were close.

**FIGURE 2** The average Raman intensity at the spectral regions around 537, 829, 937, and 1,051 cm\(^{-1}\) for blood samples obtained from diabetic (D), high dose (HD) and low dose (LD) herbal extract treated, pioglitazone (PG) treated, and nondiabetic (ND) rats. The red line was drawn as a guide. Intensity in all the peaks decreased with treatment and approached values for nondiabetic rats [Colour figure can be viewed at wileyonlinelibrary.com]
the LD treatment of the rats with the herbal extract. These AUC values indicate the possibility of Raman spectroscopy in performing antidiabetic drug efficacy comparative studies with AUC values of Raman spectra being one of the measurement parameters.

3.2 | Principal component analysis

PCA was used to assess the power of Raman spectroscopy in spectrally differentiating between the rats (diabetic, nondiabetic, those treated with low- and high-dose herbal extract, treated with pioglitazone and normal/nondiabetic).

Figure 4 shows score plots of PC1 versus PC2, PC1 versus PC3, and PC2 versus PC3 with respective percentage variance shown (in brackets). The three PCs accounted for 65% explained variance in the spectral data set. The scree plot in Figure S4 also shows why these first three PCs are more significant than the others as they carry more information about the spectral data set (each of their explained percentage variance was greater than 10%). The upper panel in Figure 4 shows that Raman spectral profiles from blood of diabetic rats (red dots) were significantly different from those of nondiabetic (black dots). This further showed the potentiality of Raman spectroscopy in performing sensitive and rapid diabetes mellitus type II detection or diagnosis in a large population. The lower panel shows that the spectral profiles from rats administered antidiabetic herbal extracts (blue and green dots), pioglitazone (cyan dots), and nondiabetic rats were similar hence no significant segregation. This was supported by the obtained variance values (from Student t test) displayed in Table 3. The bands that played a key role in the segregation between the treated from the diabetic rats are displayed in the loadings plot displayed in Figure S5b. These bands were centred around 476, 635, 1,210, and 1,572 cm\(^{-1}\) prominent in nondiabetic and treated and those around 537, 775, 829, 918–960, and 1,051 cm\(^{-1}\) prominent in diabetic rat's blood. These same bands were also shown to have significant variance across the groups as displayed in Figure S3b. The tentative assignments of the bands are displayed in Table 1. The PCA results indicated two
things, first, that the antidiabetic effects of the herbal extract *Rotheca myricoides Hochst* were comparable with those of the commercially available drug pioglitazone, and second, it showed that Raman spectroscopy can be utilized in comparative efficacy studies between new antidiabetic drugs the commercially available conventional variants as was also shown in Birech et al.[12]

4 | CONCLUSION

The work studied utility of Raman spectroscopy in detecting diabetes and comparing efficacy of herbal extract *Rotheca myricoides Hochst* in treating diabetes. It was noted that Raman spectral profiles of blood from streptozotocin-induced diabetic SD rats could be reproduced from sum of spectra from solids of creatine monohydrate, valine, leucine, isoleucine, glucose, and fructose and so showing that their levels are highly modified in diabetic rats. Raman biomarker bands for presence of diabetes mellitus type II condition were found in diabetic rats. The bands have the potential of being used as reference during diabetes screening. The intensities of the identified bands decreased in diabetic rats administered antidiabetic herbal extracts and the often used commercially available antidiabetic drug called pioglitazone. Statistical analyses together with evaluation of average intensities of the biomarker bands showed that the herbal extract *Rotheca myricoides Hochst* had greater antidiabetic effect at low dose (50 mg/kg of body weight) than in high dose (100 mg/kg of body weight). A similar result was observed with AUC values.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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Additional supporting information may be found online in the Supporting Information section at the end of the article.

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