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Drug Discovery

Glycaemic and Biochemical Changes in Response to *Syzygium Aromaticum* Administration in Diabetic Rats

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To Cite:
Ogadinma IN, Chuemere AN, Crownwell OS. Glycaemic and Biochemical Changes in Response to *Syzygium Aromaticum* Administration in Diabetic Rats. *Drug Discovery* 2026; 20: e10dd3067 doi: https://doi.org/10.54905/dissi.v20i45.e10dd3067

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Peer-Review History
Received: 30 June 2025
Reviewed & Revised: 07/August/2025 to 25/March/2026
Accepted: 07 April 2026
Published: 12 April 2026

Peer-Review Model
External peer-review was done through double-blind method.

Drug Discovery
pISSN 2278–540X; eISSN 2278–5396

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ABSTRACT

This study assessed the glycaemic and biochemical changes in response to *Syzygium aromaticum* administration in diabetic rats. The animals were sampled into 6 groups of 5 rats each. Group A; control, Group B; diabetic group, Group C; clove only, Group D-F; clove + alloxan, with concentration of 0.5,1 and 1.5 ml of clove, respectively. Diabetes was induced in groups B-F. Blood sample was collected via intraocular puncture weekly for analysis of fasting blood glucose (FBG) level. At the end of 4 weeks, the animals were sacrificed and blood samples taken for estimation of markers for oxidative stress, lipids and liver function markers. Results were significant, statistically, at $P<0.05$. The results showed the diabetic rats exhibited elevated levels of oxidative stress-induced lipid peroxidation and protein carbonylation, elevated levels of liver function markers and dyslipidemia. There was elevated FBG 1 and 2 and HbA1c, indicating poor glycemic control. When compared to the control, the treatment groups, D, E and F, dose-dependently showed a reduction in lipid peroxidation and protein carbonylation and significant increase in the levels of oxidative stress enzyme markers, decrease in ALT and AST levels and increase in HDL. There was a significant reduction in FBG 2 and HbA1c. The glycemic and biochemical effect of *S. aromaticum* may be therapeutically beneficial in alloxan-induced diabetes in Wistar rats.

Key words: Diabetes; Hyperglycemia; Cardiovascular; Lipoproteins; Biochemical

1. INTRODUCTION

In the last 40 years, there has been a reduction in the mortality rate associated with diabetes and diabetes-related heart diseases (ADA, 2021). Both diabetic and non-diabetic patients have seen their cardiovascular complications decrease by about 70% (ADA, 2020). Medical practitioners attribute this positive trend to significant progress in identifying and managing known cardiac risk factors, as well as advancements in interventional cardiology (AHA, 2021). Over recent decades, improvements in therapeutic approach have contributed to decreased mortality rates and overall enhancements of quality of life among diabetes patients (Arnett et al., 2019). Diabetes is a metabolic disease that affects the body's ability to regulate blood sugar levels,

leading to severe damage not only to the heart but also blood vessels, kidneys, eyes and nerves (Pociot and Lernmark, 2016). Heart disease constitutes various underlying health issues that impact the human heart (Ismail and Bulent, 2017). The term "cardiovascular disease" is similar but includes all types of heart disease, stroke, and blood vessel disease (Sharma et al., 2020). Coronary artery disease been the most common cardiovascular disease (Chatterjee and Kundu, 2012; Fryar et al., 2012). Coronary artery disease, a disease of a cardiac blood vessel (also called CAD) occurs when the walls of the blood vessels of the heart, known as the coronary arteries (Sharma et al., 2020), become clogged with an organic compound called plaque which builds up (Preis et al., 2005). This occlusion can restrict respiratory gases and blood flow to the heart tissues. This condition is called atherosclerosis, a disease condition, with symptoms such as hardening of the arteries (Wallace et al., 2011; Preis et al., 2005). Decreased blood flow to the heart can cause a heart attack (Fryar et al., 2012). Diabetes is a long-term metabolic disorder characterized by high blood sugar and disrupted lipid metabolism that can trigger various complications, including diabetic cardiomyopathy (Sowers et al., 2008). This condition involves structural changes in the heart and its functions leading to dysfunctionality (Wallace et al., 2011). It is an area of growing interest for researchers; to evaluate natural remedies like herbs, spices, and plant extracts as potential therapies for managing the illness alongside its consequential complications (Sung and Park, 2012). Liver enzymes can be used as a marker for insulin resistance which is a key feature for type 2 diabetes (Szkudelski, 2001; Lenzen, 2008). Elevated liver enzymes have also been linked to an increased risk of cardiovascular disease, which is a major complication in diabetes. *Syzygium aromaticum*, also called 'Clove', has been traditionally associated with some medicinal benefits (Rao, 2017). Researchers have identified several bioactive compounds in *S. aromaticum* including eugenol that possesses antioxidant (Venkatesh et al., 2020), anti-inflammatory (Sung and Park, 2012), and antihypertensive properties (Sultana et al., 2013). Some of these medicinal importance of *S. aromaticum* has deemed it necessary to investigate, further, its role on blood glucose as well as other biochemical changes. This study aimed to investigate the glycaemic and biochemical changes in response to *Syzygium aromaticum* administration in alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1. Ethical consideration

This study was approved by the Ethical Considerations Committee, Directorate of Research and Human Development, Madonna University, Elele, Rivers State, Nigeria and it is in compliance with the regulations for experimental animal use and care, as stipulated by the University. The reference Number is MUECC/20230107.

2.2. Plant collection

The buds of S, aromaticum used for this study were obtained from the botanical garden of Department of Pharmacognosy, Faculty of Pharmacy, in Madonna University, Elele, Rivers State, Nigeria. The plants were identified and authenticated by Dr. Adeleke of Pharmacognosy Department, Faculty of Pharmacy. The reference number assigned is MUN/PHARM/H/2023/076.

2.2.1. Phytochemical screening

The Phytochemical Screening of different constituents of *S. aromaticum* was done by Gas Chromatography Coupled to flame ionization detector (GCFID), using standard laboratory procedure by Lee and Harnly, (2005). This screening was used to analyze composition of volatile organic compounds (VOCs) in samples. It uses a Gas chromatograph, flame ionization detector and capillary column with a non-polar stationary phase.

2.3. Induction of Diabetes

The induction and confirmation of diabetes in rats involved a comprehensive and well-established protocol by Johnson et al., (2018). Alloxan monohydrate was dissolved in a sterile saline solution (0.9% NaCl) to prepare a 15mg/ml stock solution. The route of administration was intraperitoneal and was based on the animal's body weight after 12 hours overnight fast. To assess the development of diabetes, the blood glucose level was monitored in intervals of 2-3 days.

2.4. Animal collection

A total of thirty (30) male Wistar rats, weighing between 180-200g were used for this study. The animals were kept in the animal house of the Department of Human Physiology, Madonna University, Elele campus, for two weeks to acclimatize. The animals were grouped in six (6) cages with five (5) rats in each. The animals were kept under room temperature and exposed to 12/12 hours light and dark

cycles. The animals were grouped into control and test groups and housed in sanitized aluminum cages with bedding. They were also fed with standard rat feed as diet and distilled water ad libitum. Their cages were cleaned daily to avoid infection due to coprophagy.

2.5. Study design

The study design is presented in table 1.

Table 1. Study design

Groups	Treatment
Group A	Feed and distilled water
Group B	Diabetic
Group C	S aromaticum 1.0ml
Group D	Diabetic+ *S. aromaticum* 0.5ml
Group E	Diabetic+ *S. aromaticum* 1.0ml
Group F	Diabetic+ *S. aromaticum* 1.5ml

2.6. Administration of Syzygium aromaticum

A measured quantity equal to 50 grams Syzygium aromaticum powder was dissolved in 1 liter of water to create a homogeneous mixture that was then administered to the rats orally using an orogastric cannula. This method of treatment admini ensured direct and accurate ingestion of the Syzygium aromaticum powder mixture to the alimentary canal or gastrointestinal tract of the wistar rats, which allows for optimal digestion, absorption and likely evaluation of its therapeutic tendencies. By using the orogastric cannula or tube, precise control and adjustments over the administration process was possible, minimizing any likely variations or errors and ensuring consistency in the dosage administered to each rat. This sophisticated and standardized method allows for reliable investigation into the potential effects of the Syzygium aromaticum powder on the rats' physiological responses and health outcomes.

2.7. Biochemical tests

2.7.1. Fasting Blood Glucose (FBG)

Blood sample was collected through intra-ocular puncture. Blood glucose test was done using procedures by Sharma et al., (2014). Assaying fasting blood glucose levels involves measuring the concentration of glucose in a blood sample collected after a given period of food deprivation or fasing. Fasting blood glucose (FBG) measurement clearly and usually employs some enzymatic methods based on the reaction of glucose with glucose oxidase (GOD) or glucose dehydrogenase (GDH). These enzymes catalyze the oxidation of glucose, producing hydrogen peroxide (H_2O_2). The generated H_2O_2 is then quantified using a colorimetric or electrochemical detection system, which allows for the determination of glucose concentration.

2.7.2. Glycated hemoglobin (HbA1c)

Glycated hemoglobin was assayed in blood sample using the procedure by Klonoff et al., (2020) The principle of the assay is based on the fact that glucose molecules can non-enzymatically bind to hemoglobin in the red blood cells. The binding occurs on the N-terminal valine of the beta chain of hemoglobin, forming a stable adduct known as HbA1c. The level of HbA1c in the blood is directly proportional to the average blood glucose concentration over the lifespan of the red blood cells.

2.8. Test for oxidative stress markers

All the animals were anaesthetized using chloroform before been placed in supine position. Subsequently, an incision was made in the thoracic region of the animals, the heart tissue samples were carefully separated and collected following appropriate biomedical surgical techniques, while ensuring aseptic conditions during the procedure to avoid possible contamination. Careful extraction of the heart tissue was performed using only sterile instruments to preserve tissue integrity. Proper storage conditions, such as ultra-low temperature freezers were used to maintain sample stability. The oxidative stress markers were assayed using the following assay protocols and principles.

2.9. Assays

2.9.1. Assay for Superoxide dismutase (SOD)

This protocol is based on Chong et al., (2017) method and can be adapted depending on specific requirements and sample types. The SOD assay measures the activity of superoxide dismutase (SOD) by inhibiting the autoxidation of adrenaline. Superoxide anions produced during adrenaline oxidation can turn it into adrenochrome, but SOD prevents this reaction by converting superoxide anions into hydrogen peroxide. The amount of adrenochrome formed is directly proportional to the SOD activity.

2.9.2. Assay for Catalase (CAT)

This protocol is based on Chin-Chan et al., (2015) method and can be adapted depending on specific requirements and sample types. The assay of Catalase (CAT) activity is based on the measurement of the decomposition of hydrogen peroxide (H_2O_2) by CAT. CAT catalyzes the breakdown of H_2O_2 into water and molecular oxygen. The rate of decomposition of H_2O_2 can be measured spectrophotometrically by monitoring the decrease in absorbance at a suitable wavelength, directly indicating the CAT activity.

2.9.3. Assay for Glutathione peroxidase (GPx)

This protocol is based on Roberts et al., (2010) method and can be adapted depending on specific requirements and sample types. The assay for Glutathione Peroxidase (GPx) activity is based on the measurement of the rate of glutathione (GSH) oxidation by hydrogen peroxide (H_2O_2) in the presence of GPx. GPx catalyzes the reduction of H_2O_2 and organic hydroperoxides using GSH as a cofactor, regenerating GSH in the process. The decrease in absorbance resulting from the oxidation of GSH can be measured spectrophotometrically and is directly related to GPx activity.

2.9.4. Assay for Glutathione reductase (GR)

This protocol is based on Chatterjee et al., (2009) method. This assay evaluates GR activity by measuring or analyzing the luciferase level of expression. It is a commonly adopted method for investigating GR activation and screening likely modulators of GR activity. The assay utilizes a reporter gene that is usually regulated by GR. In this study, the reporter gene was a firefly luciferase gene under the control of a GRE (glucocorticoid response element). Binding of GR to the GRE leads to the activation of luciferase gene expression, which can be quantified easily. The GR activity is determined by measuring luciferase activity in response to different concentrations of glucocorticoids or other compounds.

2.9.5. Assay for Reduced Glutathione (GSH)

This protocol is based on Ellman (1959) method and can be adapted depending on specific requirements and sample types. The assay for Glutathione (GSH) is based on the reaction of GSH with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) to form a yellow colored compound, 5-thio-2-nitrobenzoic acid (TNB), which can be measured spectrophotometrically. GSH is a crucial antioxidant and plays a significant role in cellular redox balance.

2.9.6. Assay for Malondialdehyde (MDA)

This protocol is based on Esterbauer & Cheeseman, (1990) method and can be adapted depending on specific requirements and sample types. The assay of Malondialdehyde (MDA) is clearly based on the biochemical reaction of MDA with a compound, thiobarbituric acid (TBA), in an acidic condition, to present a chromophore that can be determined spectrophotometrically. MDA is a reactive carbonyl compound and a product of lipid peroxidation, making it a commonly used marker for oxidative stress and lipid damage.

2.9.7. Assay for Protein carbonyl (PC)

This protocol is based on Chong et al., (2017) method. The assay for protein carbonyl content involves the detection of carbonyl groups that are formed on proteins through oxidative damage. Carbonyl groups are reacted with a suitable reagent, such as 2,4-dinitrophenylhydrazine (DNPH), to form stable protein hydrazones. The hydrazones are then measured spectrophotometrically, and the amount of protein carbonyl content is quantified.

2.10. Assay for lipid profile markers

2.10.1. Assay for High density lipoprotein (HDL)

A principle and procedure for an assay to measure high-density lipoprotein (HDL) cholesterol levels, based on the study by Burstein et al., (1970). This assay for HDL cholesterol adopts the biochemical isolation of HDL particles from sampled serum through precipitation reaction using a polyanion, followed by the careful enzymatic determination of cholesterol in the isolated HDL fraction using the enzymes; cholesterol esterase and cholesterol oxidase. The enzymatic reaction produces a colored product that can be quantified and is proportional to the HDL cholesterol concentration.

2.10.2. Assay for Low density lipoprotein (LDL)

A principle and procedure for an assay to quantify the low-density lipoprotein (LDL) cholesterol levels, as based from a study by Friedewald et al., (1972). The assay for LDL cholesterol utilizes a mathematical calculation derived from the Friedewald equation, which determines or estimates the concentration of LDL cholesterol adopting measurements of total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides (TAG). This equation is based on the assumption that very low-density lipoprotein (VLDL) cholesterol can be determinated by measuring triglycerides, allowing for the indirect estimation of LDL cholesterol.

2.10.3. Assay for Total Cholesterol (TC)

A principle and procedure for an assay to measure Total Cholesterol (TC) cholesterol levels, based on the study by Burstein et al., (1970). The method used was the Enzymatic end-point method. The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4- aminoantipyrine in the presence of phenol and peroxidase.

2.10.4. Assay for Total glycerol (TG)

A principle and procedure for an assay to measure TG (triglycerides)levels, based on the study by Karlsson et al., (2012). The principle of a TG assay involves the enzymatic hydrolysis of triglycerides into glycerol and fatty acids, followed by the measurement of glycerol concentration. This is typically done using a colorimetric or enzymatic method.

2.11. Assay for liver function markers

2.11.1. Assay for Alanine transaminase (ALT)

An assay for ALT (Alanine Aminotransferase), also known as SGPT, measures the concentration of ALT enzyme in the blood based on Bergmeyer et. al., (1984) method. The principle of the ALT assay involves the measurement of the ALT enzyme activity. The ALT enzyme catalyzes the transfer of an amino group from alanine to alpha-ketoglutarate, resulting in the formation of pyruvate and glutamate. The rate of this enzymatic reaction is directly proportional to the ALT activity in the sample.

2.11.2. Assay for Aspartate transaminase (AST)

An assay for AST (Aspartate Aminotransferase), also known as SGOT, measures the concentration of AST enzyme in the blood also following the method by Bergmeyer et al., (1984). The principle of the AST assay involves the measurement of the AST enzyme activity. The AST enzyme catalyzes the transfer of an amino group from aspartate to alpha-ketoglutarate, resulting in the formation of oxaloacetate and glutamate. The rate of this enzymatic reaction is directly proportional to the AST activity in the sample.

2.12. Statistical method

The statistical package used to analyze the data was IBM-SPSS®. The results are presented as Mean Standard error of mean (SEM). The results were statistically significant at 95% confidence interval. Percentage change (%c) was calculated using method by Ilochi et al., (2019).

3. RESULTS

3.1. Phytochemical Analysis of Syzygium aromaticum

From table 2, the most predominant phytochemical in *S. aromaticum* is Eugenol. Others include sesquiterpenes, campesterol glycoside and flavonones. From table 3, the most abundant mineral in is calcium. Others present include manganese and potassium.

Table 2. Gas chromatography coupled to flame ionization detector (GCFID) analysis of *S. aromaticum*

Component	Concentration	Unit
Vanillin	11.0171	ug/ml
Crategolic acid	9.6327	ug/ml
Stigmasterol	4.4522	ug/ml
Eugenitin	16.7415	ug/ml
Flavonoids	10.87066	ug/ml
Triterpenoids	2.1012	ug/ml
Sesquiterpenes	24.7130*	ug/ml
Campesterol	4.8922	ug/ml
Phenol	14.9987	ppm
Gallotannic acid	10.3131	ug/ml
Flavonones	17.2127*	ppm
Steroids	3.4631	ppm
Kaempferol	8.0167	ug/ml
Flavone	4.100	ppm
Lectin	12.0312	ug/ml
Eugenol	24.9050*	ug/ml
Campesterol glycoside	19.0838*	ug/ml
Oxalate	13.2271	ug/ml
Catechin	1.2909	ug/ml
Oleanolic acid	2.2341	ug/ml

Key: *=Predominant phytochemicals in *S. aromaticum*

Table 3. Minerals in *S. aromaticum*

Minerals	Amount (mg/kg)
Iron	92.12
Potassium	118.17
Copper	3.162
Calcium	128.30
Magnesium	43.65
Manganese	120.82
Zinc	4.10

Table 4. fasting blood glucose changes in response to treatments

Groups	FBG 1(mg/dL)	FBG 2(mg/dL)
Control	3.26±0.82	3.28±0.82
Diabetic	4.10±0.07	8.86±1.29^{a,c}
***S. aromaticum* 1.0ml**	4.18±0.09	5.10±0.95^{b}
***S. aromaticum* 0.5m + Diabetic**	4.22±0.58	5.90±0.61^{a,b}
***S. aromaticum* 1.0m+Diabetic**	4.14±0.07	4.42±0.11^{b}
***S. aromaticum* 1.5ml+ Diabetic**	4.12±0.9	4.40±0.45^{b}
Total	24.02	31.96
Average	4.00	5.33

Key: ^{a}(P≤0.05)-Significantly different compared to control group, ^{b} (P<0.05)-Significantly different compared to Diabetic group (P≤0.05)-Significantly different compared to *S. aromaticum* 1.0ml group. FBG- fasting blood glucose. FBG 1= Fasting Blood Glucose 1, FBG 2= Fasting Blood Glucose 2.

From table 4, results showed a significant increase in FBG 2 levels in diabetic group and *S. aromaticum* 0.5ml + Diabetic when compared to the control and a significant decrease (P≤0.05) in group A(control), C(*S. aromaticum* 1.0ml), D(*S. aromaticum* 0.5ml+ Diabetic), E(*S. aromaticum* 1.0ml + Diabetic), and F(*S. aromaticum* 1.5ml + Diabetic) when compared to diabetic group. In comparison with *S. aromaticum* 1.0ml. FBG 2 levels in group B(Diabetic) and control were significantly increased.

Table 5. Percentage change in fasting blood glucose in response to treatments

Groups	FBG1(mg/dL)	%c*	FBG2(mg/dL)	%c*
Control	3.26±0.82	-	3.28±0.82	-
Diabetic	4.10±0.07	25.77	8.86±1.29*	170.12
***S. aromaticum* 1.0ml**	4.18±0.09	28.22	5.10±0.95	55.49
***S. aromaticum* 0.5ml+ Diabetic**	4.22±0.58	29.45	5.90±0.61*	79.88
***S. aromaticum* 1.0ml +Diabetic**	4.14±0.07	26.99	4.42±0.11	34.76
***S. aromaticum* 1.5ml+ Diabetic**	4.12±0.9	26.38	4.40±0.45	34.15

Key; FBG1= Fasting Blood Glucose 1, FBG2= Fasting Blood Glucose 2, (%c)= Percentage Change compared to control. Asterisk (*) indicates statistical significance compared to FBG1. (P≤0.05)= Significantly different.

From table 5, *S. aromaticum* progressive decrease in blood glucose level in correspondence with the percentage change. At 1ml and 1.5ml, *S. aromaticum* showed no significant change in fasting blood glucose in comparison to control.

Table 6. changes in glycated hemoglobin in response to treatments

Groups	HbA1c (%)
Control	2.66±0.67
Diabetic	8.22±1.26[a,c]
S. aromaticum 1.0ml	4.44±1.17[b]
S. aromaticum 0.5ml + Diabetic	5.12±0.49[a,b]
S. aromaticum 1.0ml + Diabetic	3.88±0.46[b]
S. aromaticum 1.5ml + Diabetic	3.36±0.51[b]

Key: [a](P≤0.05)-Significantly different compared to control group, [b](P≤0.05)-Significantly different compared to Diabetic group [c](P≤0.05)-Significantly different compared to *S. aromaticum* 1.0ml group

Table 6 results showed a significant increase in HbA1c levels in diabetic group D(*S. aromaticum* 1.0ml + Diabetic), when compared to the control and a significant decrease in group A(control), C (*S. aromaticum* 1.0m), D (*S. aromaticum* 0.5ml + Diabetic), E(*S. aromaticum* 1.0ml + Diabetic), and F(*S. aromaticum* 1.5ml + Diabetic) when compared to diabetic group. In comparison with *S. aromaticum* 1.0ml, HbA1c in group B (Diabetic) were significantly increased.

Table 7. Changes in lipid profile markers in response to treatments

Groups	HDL(mg/dL)	LDL(mg/dL)	TC (mg/dL)	TG(mg/dL)
Control	6.60±1.67	2.12±0.53	1.88±0.48	1.76±0.47
Diabetic	3.08±1.26[a,c]	4.12±0.51[a,c]	5.14±0.59[a,c]	3.76±0.44[a,c]
S. aromaticum 1.0ml	9.68±2.13[a,b]	1.26±0.74[b]	2.26±0.89[b]	1.78±0.58[b]
S. aromaticum 0.5ml+Diabetic	8.74±0.99[b]	2.40±0.56[b]	1.64±0.14[b]	2.98±0.48
S. aromaticum 1.0ml +Diabetic	9.64±0.61[b]	1.88±0.15[b]	1.64±0.07[b]	2.08±0.29[b]
S. aromaticum 1.5ml +Diabetic	10.90±0.10[a,b]	1.28±0.86[b]	1.36±0.04[b]	1.36±0.60[b]

Key: [a](P≤0.05)-Significantly different compared to control group, [b](P≤0.05)-Significantly different compared to Diabetic group [c](P≤0.05)-Significantly different compared to *S. aromaticum* group. Data represented as Mean ±SEM. P≤0.05 was considered significant.

Results showed (Table 7) a significant increase in HDL levels in group C(*S. aromaticum* 1.0ml) and F(*S. aromaticum* 1.5ml + Diabetic) when compared to the control and a significant increase (P≤0.05) in group A(control), C(*S. aromaticum* 1.0ml), D(*S. aromaticum* 0.5ml +

Diabetic), E(*S. aromaticum* MD + Diabetic), and F(*S. aromaticum* 1.5ml+ Diabetic) when compared to diabetic group. A significant decrease in HDL levels in Group B(Diabetic) when compared to control, was also shown in the result. In comparison with *S. aromaticum* 1.0ml, HDL levels in group B(Diabetic) and control were significantly decreased. There was a significant increase in LDL levels in diabetic group when compared to the control and a significant decrease in group A(control), C (S. aromaticum 1.0 ml), D (*S. aromaticum* 0.5ml + Diabetic), E(*S. aromaticum* 1.0ml + Diabetic), and F(*S. aromaticum* 1.5ml + Diabetic) when compared to diabetic group. In comparison with *S. aromaticum* 1.0ml, LDL levels in group B(Diabetic) were significantly increased. There was a significant increase in TC levels in the diabetic group when compared to the control and a significant decrease in group A(control), C (*S. aromaticum* 1.0ml), D (*S. aromaticum* 0.5ml + Diabetic), E(*S. aromaticum* 1.0ml + Diabetic), and F(*S. aromaticum* 1.5ml + Diabetic) when compared to diabetic group. In comparison with *S. aromaticum* 1.0ml, TC levels in group B (Diabetic) were significantly increased.

Results showed a significant increase in TG levels in diabetic group when compared to the control and a significant decrease in group A(control), C(*S. aromaticum* 1.0ml), E(*S. aromaticum* 1.0ml + Diabetic), and F(*S. aromaticum* 1.5ml + Diabetic) when compared to diabetic group. In comparison with *S. aromaticum* 1.0ml, TG levels in group B(Diabetic) were significantly increased.

Table 8. changes in oxidative stress markers in response to treatments

Groups	SOD(U/g)	CAT(U/g)	GPx(U/g)	GR(U/g)
Control	8.54±2.17	2.28±0.57	1.76±0.45	1.94±0.50
Diabetic	4.14±1.73[a, c]	1.62±0.30[a, c]	0.86±0.34[c]	0.90±0.33[a c]
***S. aromaticum* 1.0ml**	10.44±2.21[ab]	4.86±0.92[a],[b]	5.94±1.29[ab]	3.72±0.80[a,b]
***S. aromaticum* 0.5ml+Diabetic**	4.14±2.00[a c]	2.68±0.74[c]	3.08±1.13[c]	2.34±0.57[bc]
***S. aromaticum* 1.0ml +Diabetc**	8.68±1.42	2.76±0.21[c]	3.46±0.43[b, c]	2.44±0.12[bc]
***S. aromaticum* 1.5ml+Diabetic**	10.94±0.34[ab]	4.18±0.27[a,b]	5.32±0.61[a,b]	3.38±0.17[a,b]

Key: [a](P≤0.05)-Significantly different compared to control group, [b] (P≤0.05)-Significantly different compared to Diabetic group [c](P≤0.05)-Significantly different compared to *Syzygium aromaticum* group. SOD-superoxide dismutase, CAT-catalase, GPx- Glutathione peroxidase, GR-glutathione reductase.

From table 8, results showed a significant increase in SOD levels in group C (*S. aromaticum* 1.0ml) and F (*S. aromaticum* 1.5ml+ Diabetic) when compared to the control and diabetic group. A significant decrease in SOD levels in Group B(diabetic) and D(*S. aromaticum* 0.5ml + Diabetic) when compared to control was also shown in the result. In comparison with *S. aromaticum* 1.0ml only group SOD levels in group B(diabetic) and D (*S. aromaticum* 0.5ml + Diabetic) were significantly decreased, but significantly increased in group F (S. aromaticum 1.5ml+ Diabetic).

Still from table 8, Results showed a significant increase in CAT levels in group C (*S. aromaticum* 1.0ml) and F (*S. aromaticum* 1.5ml + Diabetic) when compared to the control and diabetic group. A significant decrease in CAT levels in Group B (diabetic) and group D (*S. aromaticum* 0.5ml + Diabetic) when compared to control was also shown in the result. In comparison with S. aromaticum 1.5ml only group, CAT levels in group B, D, E and F were significantly decreased.

Results showed a significant increase in GPx levels in group C (*S. aromaticum* 1.0ml) and F (*S. aromaticum* 1.5ml+ Diabetic) when compared to the control. A significant increase in group C (*S. aromaticum* 1.0ml), E(*S. aromaticum* 1.0ml+ Diabetic) and F (*S. aromaticum* 1.5ml + Diabetic) group when compared to diabetic group was also shown in the result. In comparison with *S. aromaticum* 1.0ml only group, GPx levels in group B, Dand E were significantly decreased.

Results showed a significant increase in GR levels in group C (*S. aromaticum* 1.0ml only) and F (*S. aromaticum* 1.5ml+ Diabetic) when compared to the control and a significant increase in group C(*S. aromaticum* 1.0ml), D(*S. aromaticum* 0.5ml + Diabetic), E(*S. aromaticum* 1.0ml+ Diabetic) and F(*S. aromaticum* 1.5ml+ Diabetic) in diabetic group. A significant decrease in group B(diabetic), when compared to control was also shown in the result. In comparison with *S. aromaticum* 1.0ml only group, GR levels in group B, D, and E were significantly decreased.

From table 9, results showed a significant increase in GSH levels in group C(S. aromaticum 1.0ml), E (*S. aromaticum* 1.0ml + Diabetic) and F(*S. aromaticum* 1.5ml + Diabetic) when compared to the control and a significant decrease in group A(control), C(*S. aromaticum* 1.0ml), E(*S. aromaticum* 1.0ml + Diabetic), and F(*S. aromaticum* 1.5ml + Diabetic) when compared to diabetic group. A significant decrease in GSH levels in Group B(Diabetic) when compared to control, was also shown in the result. In comparison with *S. aromaticum* 1.0ml, GSH levels in group B(Diabetic) were significantly decreased.

Still from table 9, results showed a significant decrease in MDA levels in group B(Diabetic) and C (*S. aromaticum* 1.0ml) when compared to the control. A significant decrease in MDA levels in Group B(Diabetic), A(control), C(*S. aromaticum* 1.0ml), D(*S. aromaticum*

0.5ml + Diabetic), E(*S. aromaticum* 1.0ml + Diabetic), and F(*S. aromaticum* 1.5ml + Diabetic) when compared to diabetic group, was also shown in the result. In comparison with *S. aromaticum* 1.0ml, MDA levels in control group were significantly decreased and significantly increased in diabetic group.

Table 9. changes in oxidative stress markers in response to treatments

Group	GSH(U/g)	MDA(mmol/g)	PC(mmol/g)
Control	1.30±0.34	1.18±0.30	0.50±0.14
Diabetic	0.42±0.20 [a,c]	7.66±1.66[a,c]	2.60±0.53[a,c]
***S. aromaticum* 1.0ml only**	4.20±1.03 [ab]	2.32±1.97[a b]	0.76±0.61[a,b]
***S. aromaticum* 0.5ml + Diabetic**	2.54±0.68	1.60±0.41 [b]	2.26±0.57[a,c]
***S. aromaticum* 1.0ml + Diabetic**	3.42±0.56 [a,b]	1.40±0.15 [b]	0.66±0.19[b]
***S. aromaticum* 1.5ml + Diabetic**	4.76±0.17 [a,b]	1.16±0.40 [b]	0.36±0.40[b]

Key: [a](P≤0.05)-Significantly different compared to control group, [b] (P≤0.05)-Significantly different compared to Diabetic group, [c] (P≤0.05)-Significantly different compared to *Syzygium aromaticum* group. GSH- reduced glutathione, MDA- malondialdehyde, PC-protein carbonyl.

Results showed a significant increase in PC levels in group B(Diabetic), C(*S. aromaticum* 1.0ml) and D(*S. aromaticum* 0.5ml + Diabetic) when compared to the control. A significant decrease in group A(control), C(*S. aromaticum* 1.0ml), E(*S. aromaticum* 1.0ml + Diabetic), and F(*S. aromaticum* 1.5ml + Diabetic) when compared to diabetic group, was also shown in the result. In comparison with *S. aromaticum* 1.0ml, PC levels in group were significantly increased in diabetic group and group D(*S. aromaticum* 0.5ml + Diabetic) but were significantly decreased in control group.

Table 10. changes in liver function markers in response to treatments

Groups	ALT(U/L)	AST(U/L)
Control	10.10±2.53	6.36±1.60
Diabetic	24.12±2.93[a,c]	13.06±1.33[a,c]
S. aromaticum 1.0ml	11.56±4.14[b]	5.14±1.77[b]
***S. aromaticum* 0.5ml + Diabetic**	11.46±1.70[b]	8.24±1.32[b]
***S. aromaticum* 1ml + Diabetic**	10.48±0.57[b]	6.64±0.65[b]
***S. aromaticum* 1.5ml + Diabetic**	8.72±0.26[b]	3.90±0.11[b]

Key: [a](P≤0.05)-Significantly different compared to control group, [b](P≤0.05)-Significantly different compared to Diabetic group [c] (P≤0.05)-Significantly different compared to *Syzygium aromaticum* group. ALT- alanine transaminase, AST-aspartate transaminase.

From table 10, the results showed a significant increase in ALT levels in diabetic group when compared to the control and a significant decrease in group A(control), C(*S. aromaticum* 1.0ml only), D(*S. aromaticum* 0.5ml + Diabetic), E (*S. aromaticum* 1.0ml + Diabetic), and F(*S. aromaticum* 1.5ml + Diabetic) when compared to diabetic group. In comparison with *S. aromaticum* 1.0ml, ALT levels in group B(Diabetic) and control were significantly increased.

Results showed a significant increase in AST levels in diabetic group when compared to the control and a significant decrease in group A(control), C(*S. aromaticum* 1.0ml), D(*S. aromaticum* 0.5ml + Diabetic), E(*S. aromaticum* 1.0ml + Diabetic), and F(*S. aromaticum* 1.5ml + Diabetic) when compared to diabetic group. In comparison with *S. aromaticum* 1.0ml, AST levels in group B(Diabetic) and control were significantly increased.

4. DISCUSSION

In this study, phytochemical screening was done to identify, verify and also quantify some important bioactive compounds present in S. aromaticum. These screening or analytic investigation provided some valuable insights into the therapeutic and nutraceutical composition of the plant and helped explain its potential cardioprotective effects. The highest components identified, as reported, in this study were eugenol, sesquiterpenes, campesterol glycoside and flavonones. Eugenol, the major component, is a phenolic compound known for its antioxidant and anti-inflammatory properties (Han et al., 2019). It has been extensively studied for its health-promoting effects, showing potential in cardiovascular disease prevention and management (Sharma et al., 2020). Eugenol substitutes

as a good free radical scavenger and, as well, inhibits the activity of some pro-inflammatory enzymes, contributing to its cardioprotective properties (Lu et al., 2020). It has been presented to reduce myocardial ischemia-reperfusion injury and improve cardiac function (Munhoz et al., 2006).

Sesquiterpenes exhibit various beneficial biological activities, including antioxidant, antimicrobial, and anti-inflammatory effects (Khoubnasabjafari et al., 2017). Sesquiterpenes have been reported to have potential cardioprotective effects due to their ability to reduce inflammation, improve endothelial function, and mitigate oxidative stress (Plomley and Best, 2002). These actions may contribute to their part in seriously preventing or fighting against cardiovascular diseases. Campesterol glycoside is a plant-based sterol compound that has been studied and reported to have some cholesterol-lowering effects and may also add to the prevention of cardiovascular diseases (CTTC et al., 2015). Their ability to lower low-density lipoprotein cholesterol (LDL) levels and also reduce atherosclerosis disease risk factors makes them a potential therapeutic agent for cardiovascular health (Chuemere et. al., 2018). Flavonones, a subclass of flavonoids, are also found in significant amounts in clove powder. Flavonones possess antioxidant properties and have been reported to have potential cardioprotective effects. They exhibit a potent free radical-scavenging or antioxidant property, modulate enzyme activity, and modulate some signaling pathways that are involved in oxidative stress and inflammatory mechanisms (Wallace et al., 2011). Flavonones have shown promising effects in mitigating oxidative stress and inflammation, both of which contribute to the development and progression of cardiovascular diseases (Manach et al., 2004). From the phytochemical analysis of this study, the presence of eugenol, sesquiterpenes, campesterol glycoside, and flavonones in *S. aromaticum*, suggests its potential therapeutic value in cardiovascular diseases, particularly in the context of diabetic cardiomyopathy. Further studies are needed to explore the specific mechanisms by which these bioactive compounds exert their cardioprotective effects and determine the optimal dosages for maximum benefit. Results from this study showed a significant decrease in glucose levels in treatment groups when compared to the diabetic group indicating the potential hypoglycemic effects of *S. aromaticum*, this may be due to the clove powder containing active compounds such as phenolic compounds and flavonoids, which may inhibit the enzymes responsible for carbohydrate digestion in the intestines, this can slow down the breakdown and absorption of carbohydrates (Watson and Pessin, 2013), Which may lead to a more progressive release of glucose into the circulation and preventing abrupt spikes in blood level of glucose. It could also be as a result of the antioxidant tendencies that clove powdered extract is known to exhibit (Nathan and Kuenen, 2008), which can help protect pancreatic beta cells, the source of insulin, from damage caused by oxidative stress modifications (Lenzen, 2008), this may lead to improved insulin secretion and better control of blood glucose levels. *S. aromaticum*, has been recognized for its potential health benefits, including its stance on glucose homeostasis; Yasmin et al., (2019) investigated the effects of clove extract on glycemic control in streptozotocin-induced diabetic rats. They observed a significant decrease in fasting blood glucose levels. These findings indicate that *S. aromaticum* may improve glycemic control and reduce long-term glucose exposure.

Lipid profile markers play a crucial role in assessing the risk and progression of cardiovascular diseases, which are often associated with diabetes mellitus (DM) complications. Altered lipid metabolism, characterized by increased total cholesterol (TC), low-density lipoprotein cholesterol (LDL), triglycerides (TG), and decreased high-density lipoprotein cholesterol (HDL) levels, contributes to the development of diabetic dyslipidemia (Sekhri et al., 2016). Syzygium aromaticum may possess potential therapeutic agents that may be able to mitigate lipid profile abnormalities in diabetes-related cardiovascular disorders. Results from this study showed a significant decrease in total cholesterol, triglycerides, and low-density lipoprotein (LDL) cholesterol levels, while increasing high-density lipoprotein (HDL) cholesterol levels in treatment groups when compared to the diabetic group. This could be as a result of hypolipidemic activity of clove powder attributed to its antioxidant properties and the presence of bioactive compounds such as phenolic compounds and flavonoids. These compounds are believed to inhibit the biosynthesis of cholesterol and triglycerides in liver, thus reducing their levels in the blood. Additionally, clove powder may increase the excretion of cholesterol through bile acid binding, further contributing to its lipid lowering effects (Patel et al., 2015). In another study, researchers examined the effects of clove extract on lipid profile markers in rats (Riyah et al., 2019). They reported that clove extract significantly reduced total cholesterol, triglycerides, and low-density lipoprotein (LDL) cholesterol levels, while increasing high-density lipoprotein (HDL) cholesterol levels. This suggests that clove may have a positive impact on lipid profile markers. Another study investigated the effects of clove extract on lipid profile markers in mice fed a high-cholesterol diet (Patel et al., 2015). The results showed that clove extract significantly lowered total cholesterol, triglyceride, and LDL cholesterol levels, while increasing HDL cholesterol levels. These findings suggest that clove may have a beneficial effect on lipid profile markers in individuals with high cholesterol levels (Reddy et al., 2010). Additionally, a study investigated the effects of clove oil on lipid profile markers in rats with high-fat diet-induced dyslipidemia (Sekhri et al., 2016). The study demonstrated that clove oil significantly reduced total cholesterol, triglycerides, and LDL cholesterol levels, while increasing

HDL cholesterol levels. These findings suggest that clove oil may have a potential lipid-lowering effect (Sekhri et al., 2016). The available studies, suggests that clove may have beneficial effects on lipid profile markers in diabetes. Clove has been shown to lower TC, LDL-C, and TG levels while increasing HDL-C levels in diabetic animal models. These effects may contribute to the improvement of dyslipidemia, which is a crucial factor in reducing the risk of cardiovascular complications associated with diabetes. Oxidative stress is a condition that arises from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms. It plays a crucial role in the development and progression of various diseases, including cardiovascular diseases. Results from this study showed a significant reduction in MDA and PC levels while increasing the activities of antioxidant enzymes such as SOD and catalase (CAT), when compared to the diabetic group, this could be as a result of the antioxidant properties found in *S. aromaticum*. *S. aromaticum* probably has a good antioxidant capacity, which may be attributed to its bioactive components, including eugenol, sesquiterpenes, campesterol glycoside, and flavonones. Eugenol, in particular, has been extensively studied for its antioxidant properties (Ibrahim et al., 2019). It acts as a free radical scavenger, inhibiting the oxidation of lipids and protecting against oxidative damage (Azman et al., 2018).

Several studies have reported the antioxidant effects of clove and its bioactive compounds in various models of oxidative stress (Usman et al., 2020; Venkatesh et al., 2020). Studies investigating the impact of *S. aromaticum* on oxidative stress markers have shown promising results. A study by Ibrahim et al., (2019) demonstrated that clove supplementation significantly decreased oxidative stress markers, including malondialdehyde (MDA), while increasing antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) in diabetic rats (Ilochi et al., 2024; Alqarni and Nişandağ, 2020). These findings suggest that clove can attenuate oxidative stress by enhancing the antioxidant defense system. In another study, Azman et al., (2018) investigated the effect of clove on oxidative stress in rats with lead-induced toxicity. The supplementation of clove extract significantly reduced MDA levels while increasing the activities of antioxidant enzymes such as SOD and catalase (CAT) (Chan et al., 2020). These results highlight the potential of clove in combating oxidative stress associated with toxic exposures. The antioxidant properties of clove and its bioactive components are believed to operate through multiple mechanisms. Eugenol has been reported to inhibit the activity of ROS-generating enzymes, such as NADPH oxidase, and increase the expression of antioxidant enzymes (Hafezi et al., 2020). Additionally, eugenol can scavenge free radicals and chelate metal ions involved in oxidative stress generation (De Sousa et al., 2019).

Sesquiterpenes found in clove exhibit both direct antioxidant effects and the ability to modulate cellular signaling pathways involved in oxidative stress (Han et al., 2019). The available literatures suggest that *S. aromaticum* has a significant impact on oxidative stress markers. Its bioactive components, including eugenol, sesquiterpenes, campesterol glycoside, and flavonones, contribute to its antioxidant properties. Clove supplementation has shown the ability to reduce oxidative stress markers, such as MDA, while increasing antioxidant enzyme activity. These effects are likely mediated through various mechanisms, including ROS scavenging, modulation of ROS-generating enzymes, and modulation of cellular signaling pathways involved in oxidative stress. Liver enzymes, when elevated, can indicate liver damage or inflammation, which is common in people with diabetes. These enzymes can also be used as a marker for insulin resistance (Szkudelski, 2001; Lenzen, 2008). alanine aminotransferase (ALT) and aspartate aminotransferase (AST), results from this study showed a significant reduction in the levels of ALT and AST, this could be due to its high levels of antioxidants such as phenolic compounds and flavonoids, it could also be as a result of its anti-inflammatory properties.

Study by Ibrahim et al., (2019) found that clove extract attenuated oxidative stress in experimental diabetic rats, leading to reduced levels of liver enzymes, including ALT and AST. Azman et al., (2018) demonstrated the cytotoxic and oxidative stress activities of clove extract on human colorectal adenocarcinoma HT29 cells. This study noted a decrease in ALT and AST levels, indicating the potential protective role of *S. aromaticum* against liver damage. Usman et al., (2020) Investigated the therapeutic tendency of clove extract on streptozotocin-induced diabetic rats and reported that it promoted antioxidant status and overall immune function. This improvement was also followed by a reduction, which was significant, in ALT and AST levels, suggesting a protective effect on liver function. Venkatesh et al. (2020) investigated the protective potential of an aqueous extract from dried flower buds of same *S. aromaticum* against isoproterenol-induced myocardial infarction in wistar rats. The study reported a drop in levels of ALT and AST, translating the potential of clove extract in protecting the liver against myocardial injury. Alqarni and Nişandağ (2020) evaluated the antioxidant potential of clove buds extract against ethylene glycol-induced renal injury in wistar rats. It was found that the extract dose-dependently and effectively reduced ALT and AST levels, indicating its potential in alleviating liver damages caused by renal injury. Chan et al., (2020) studied the cardioprotective effects of phenolic-rich extracts from *S. aromaticum* by attenuating oxidative stress-induced apoptosis. This research observed a significant reduction in ALT and AST activities in the treated group, indicating its potential in protecting liver cells from oxidative damage. Hafezi et al., (2020) investigated the effect of *S. aromaticum* hydroalcoholic

extract on histopathological changes and oxidative stress in the hearts of rats with type 2 diabetes. It was found that the extract significantly decreased ALT and AST levels, suggesting its hepatoprotective effects in the context of diabetic complications. The collective findings from various studies provide considerable evidence supporting the potential of *S. aromaticum* in reducing ALT and AST levels, indicating its hepatoprotective effects. These studies highlight the antioxidative properties of clove extract, contributing to decreased oxidative stress and improved liver health.

5. CONCLUSION

The findings of this study suggest that *Syzygium aromaticum* may have potential cardio-protective benefits in alloxan- induced diabetic wistar rats. These results have Important implications for the development of alternative therapeutic interventions or treatments for diabetic patients at risk of cardiovascular disease.

Acknowledgements

The authors greatly appreciate every individual that contributed to the success of this research, most especially Miss Omueti Shalom Crownwell, an exceptional student.

Author Contributions:

All authors carried out the research, analyzed the data, read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Ethical Approval

In this article, the animal regulations are followed as per the ethical committee guidelines of Directorate of Research and Human Development, Madonna University, Elele, Rivers State, Nigeria; the authors observed the glycaemic and biochemical changes in response to *Syzygium Aromaticum* administration in diabetic rats. The Animal ethical guidelines are followed in the study for observation, identification & experimentation. The animal ethical committee reference Number is MUECC/20230107. Also, the ethical guidelines for plants & plant materials are followed in the study for species observation, identification & experimentation. The reference number assigned is MUN/PHARM/H/2023/076.

Informed Consent

Not applicable.

Conflicts of interests

The authors declare that they have no conflicts of interest, competing financial interests or personal relationships that could have influenced the work reported in this paper.

Funding

This research did not receive any external funding like specific grant from funding agencies in the public, commercial, or nonprofit sectors.

Data and materials availability

All data associated with this study will be available based on the reasonable request to corresponding author.

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