Tropical Journal of Applied Natural Sciences

Vol. 4 Issue 1 (2026)

Tropical Journal of Applied Natural Science

ISSN: 2449-2043

https://tjansonline.org/view-paper.php?id=72;

Volume/Issue: Volume 4, Issue 1 **Published:** March 17, 2026

ASSESSMENT OF THE pH, SHORT CHAIN FATTY ACIDS AND BLOOD GLUCOSE LEVEL OF TYPE 2 DIABETIC PATIENTS ADMINISTERED WITH *NAUCLEA LATIFOLIA* FRUIT EXTRACTS

ORJI, C.C., ORJI, O.B., IHEUKWUMERE, I.H., ENWEANI, I. B., UMEAKU, C. N., ORJI, S.L., NAGOGO, H.S., YAKUBU, I.M. AND CHIGBU, C.C.

Department ofApplied Microbiology and Brewing, NnamdiAzikiwe University, Awka, Nigeria,

Department ofMicrobiology, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State

Corresponding author email: chinedu.orji@unizik.edu.ng

ABSTRACT

Studies have shown that diabetic patients are usually associated with dysbiosis. Several studies have been done on restoration ofblood glucose level but little or no study has been done on pH and short chain fatty acids level of diabetic patients in Nigeria majorly Anambra State. Hence this study focused on Assessment of the pH, short chain fatty acids and blood Glucose level of type 2 diabetic patients administered with *Nauclea latifolia* fruit extracts. A total of 40 stool samples; 20 samples from diabetic (10 males and 10 females) patients and 20 samples from non-diabetic (10 males and 10 females) subjects, were collected and analyzed for pH, short chain fatty acids (SCFA) / (butyrate and propionate) and blood glucose level using instrumentational techniques. The unique bacterial isolates encountered in the gut of diabetic patients were appropriately characterized. Phytochemical constituents of*Nauclea latifolia* fruit extracts (NLFE) was quantitatively determined using gravimetric and spectrophotometric methods; *In vivo* technique using human subjects was employed to determine the effect ofNLFE on pH, short chain fatty acid and blood glucose level. The data generated were analyzed using one-way Analysis of Variance (ANOVA) at 95% confidence level, and compared using student "t" test. There were decreased in stool pH and blood glucose, and significant ($P<0.05$) decreased in SCFA among the diabetic patients. Alkaloids, saponins, phenolics, tannins, flavonoids, steroids and glycosides were detected from NLFE. The study has revealed that NLFE showed pronounce activity and was able to restore 80% ofpH, short chain fatty acids and blood glucose level of diabetic patients.

Keywords: Dysbiosis, Diabetics, Extracts, *Nauclea latifolia*

1.0 INTRODUCTION

In the past decade, there has been growing evidence suggesting that gut dysbiosis may be a major contributor to T1D development (Vaarala, 2013). A variety of studies have identified differences in the gut microbiota of healthy subjects and T1D patients (Bibbò *et al.,* 2017). In addition, growing evidence from well-controlled intervention studies in rodent models has supported the causative association between gut dysbiosis and T1D pathogenesis. The use of probiotics, the use of antibiotics, fecal microbiota transplantation, and diet intervention are the methods commonly used in these studies to alter the composition of gut microbiota. It has been proposed that the altered intestinal microbiota may impact T1D pathogenesis by increasing gut permeability (Bosi *et al.,* 2006), facilitating intestinal inflammation (Westerholm-Ormio *et al.,* 2003), and disturbing immunological maturation (Bendtsen *et al.,* 2015, Brown *et al.,* 2019). Nevertheless, given the paucity of well-controlled studies in humans owing to the lack of corrective methods for confounding factors, gut microbiota as a causal factor leading to the progression of T1D remains speculative. Once the causative relationship between gut microbiota and T1D development is confirmed and the related pathophysiological mechanisms are delineated, the gut microbiota will be a novel area to explore for new preventative or therapeutic strategies for T1D. Discovery of a clear association between gut dysbiosis and T1D is of significant clinical importance as microbiota-based interventions such as probiotics can reduce or even prevent the burdensome requirement of injected insulin.

The 16S ribosomal RNA (rRNA) gene sequencing technique has become the most widely used method to investigate the composition of microbial ecosystems in recent years (Whelan and Surette, 2017, Mancabelli *et al.,*2017). Compared with traditional culture-based methods and previously used low-resolution methods, which can only identify specific bacteria, 16S rRNA-based sequencing as a rapid, cost-effective, and less labour-intensive microbial detection approach can analyse the composition of the whole microbial community and significantly improve the resolution of bacterial identification (Forde and O'Toole, 2013). To date, a variety of case-control studies have observed perturbed intestinal microflora in T1D patients compared

with healthy controls (HCs) using 16S rRNA gene sequencing. Given that different bacterial detection technologies have distinct levels of discrimination, which limits the ability to make accurate and specific comparisons across studies (Wright *et al.,* 2015), we performed a systematic review to provide an overview of the intestinal microflora profile in patients with T1D based on 16S rRNA gene sequencing.

Type 2 diabetes mellitus (T2DM) is a progressive metabolic disease characterized by pancreatic β-cell dysfunction and peripheral insulin resistance, leading to defects in glucose metabolism and chronic low-grade inflammation. Both genetic and environmental factors are closely related to the development of this chronic disease, and certain environmental factors, such as caloric intake, nutrient composition, ambient air pollution and physical inactivity, are important reasons for the continuous increase in its prevalence (Kahn *et al*., 2014; Kang *et al*., 2022). It was reported that more than 460 million adults worldwide had diabetes in 2019, and this number would rise to 700 million by 2045 (Saeedi *et al*., 2019). Since T2DM contributes to the occurrence of harmful macrovascular and microvascular outcomes (Sehgal *et al*., 2021; Zeng *et al*., 2021; Zhang X. *et al.*, 2021; Zhao *et al*., 2021), it not only brings physical and mental pain to patients, but also leads to a major medical economic burden. Therefore, it is of great significance to explore the novel pathogenesis and treatment of T2DM.

The early human studies showed that the pH of the gastric juice influenced growth of the bacteria in the stomach and intestine, and therefore, pH affected both the quality and quantity of the gastrointestinal microbiota. A recent study demonstrated that female NOD mice maintained on acidic drinking water developed insulitis and hyperglycemia rapidly, compared to those on neutral drinking water (Sofi *et al.,* 2014). The 16S rRNA-targeted pyrosequencing revealed a significant change in the composition and diversity of gut microbiota when the pH of drinking water was altered (Sofi *et al.,* 2014).

2.0 MATERIALS AND METHODS

Sample Collection and Transportation

Stool and blood samples were collected by sampling technique and analyzed in the Microbiology Laboratory unit of

Nnamdi Azikiwe University, Awka. Ripe fruits of *Nauclea latifolia* were collected from Ilorin, Kwara State, Nigeria. They were identified and authenticated by Prof. I.B. Enweani. The fruits were chopped into small pieces and air-dried at room temperature. They were then pulverised using a blender (Supper master blender, model: SMB-2977) to yield fine powdered material. Then, 45 g of the powdered sample was mixed with 8.5 litres of clean water, boiled for 20 minutes and allowed to cool. The resulting extract was filtered and kept refrigerated in air-tight containers. For six months, the filtered extract was given twice a week to both the diabetic patients and the healthy individuals (Enweani, 2020). Their blood glucose levels were examined morning (fasting) and night (random) for each day.

2.1 Evaluation of pH and Percentage of Short Chain Fatty Acids of Stool Samples from Diabetic and Non-diabetic Subjects

pH of the stool samples: The stool samples collected from the experimented subjects was used for this study. The stool sample was prepared in 10^{-1} diluted form by weighing 10 g of the sample into 250 mL test tube (Pyrex). This was followed by addition of 30 ml of sterile normal saline, and this was thoroughly shaken and then made up to 100 mL using the sterile normal saline. The Table top pH meter was first standard using buffers (pH = 4.0, 7.0 and 11.0), and measured the pH readings of the sample solutions (AOAC, 2019)

2.1.1 Short chain fatty acids (SCFAs): This was carried out using gas chromatographic (GC) technique as published by AOAC (2019). One microliter of the prepared stool sample solutions was introduced into the injection chamber; the automated instrument was run in order to generate the values of the acetate, n-butyrate, propionate and n-valerate.

Effect of *Nauclea latifolia* fruit extracts on pH, short chain fatty acids and blood glucose

Preparation of *Nauclea latifolia* fruit extracts: The fresh fruits of *Nauclea latifolia* was collected and it was appropriately authenticated. The fruit samples were ground to paste form using sterile electric grinder (LE Max/ LXB 242). Twenty grams of the ground sample was macerated with distilled water for 72 h. Whatman No 1 filter paper was used to filter the mixture. The extract was concentrated by evaporating to dryness at room temperature in a steady air current (Iheukwumere *et al.,* 2018). Then 40 g of the extract was

dissolved in phosphate buffer saline (PBS) and made-up to 2000 mL using the PBS in order to obtain 20 mg/mL concentration

Acute toxicity: The albino Wistar rats were monitored for 72 h for mortality cases as described in the work published by Iheukwumere *et al*. (2018). A total of 16 albino Wistar rats were used for this study. The rats were grouped into two groups. Each group contained 8 rats each. The test rats were orally administered 1.0 g/ kg (tenfold of normal administration) of the prepared extracts whereas the other group (control group) was giving ordinary distilled water as normal control. The rats in each group were monitored for 72 h during which the acute toxicity was determined after 72 h.

2.4. Determination of gut stability and glucose level: A total of 20 diabetic patients, 10 males and 10 females from different hospitals were recruited for this study. The patients that were already on drugs were excluded in this study. This was a single- blinded study. The prepared extract was orally administered by the diabetic patients, ten milliliters, two times per day, for period of 4 months. Then stool samples from each patient was collected every month and analyzed for pH, short chain fatty acids and blood glucose level as already described above.

2.5. Statistical Analysis

The data generated were analyzed using one-way Analysis ofVariance (ANOVA) at 95% confidence level, and compared using student "t" test.

3.0 RESULTS

The mean pH of the stool samples collected from diabetic and non-diabetic subjects revealed that the pH values obtained from diabetic subjects deviated from the normal pH ofhuman gut. There was decreased in pH a shown in Table 1, and these decreased were not statistically significance ($P > 0.05$). The mean pH values from the male subjects were slightly higher than that of the female.

Butyrate and Propionate were the two short chain fatty acids analyzed from the stool samples of diabetic and non-diabetic subjects. The study revealed that the respective values ofbutyrate and propionate were significantly ($P < 0.05$) reduced among the diabetic male and female patients as shown in Table 2.

The blood samples were drawn from diabetic and non-diabetic male and female subjects. The study showed that

the mean blood glucose values from diabetic male and female subjects increased above the normal standard (70 – 100 mg/dL) and these increased were not statistically significance ($P > 0.05$). There was also slight increase in blood glucose level of females above the males.

In Vitro Activity of *Nauclea latifolia* Fruit Extract in the growth of the Resistance Bacterial isolates

The phytochemical constituents of *Nauclea latifolia* fruit extract revealed the presence of alkaloids, saponins, phenolics, flavonoids, glycosides, steroids and tannins as shown in Table 4.

Effect of *Nauclea latifolia* Fruit Extract on Blood Glucose level in Diabeic Patients

The mean pH of the stool samples from the experimented subjects revealed that the pH values from the diabetic patients ranged from 5.01 – 5.11 and this showed deviation from the normal gut pH, but the pH values were progressive increased in every two months among the diabetic patients that were taking NLFE but the increased was not statistically significance ($P > 0.05$) as shown in Table 5. The progressive increased in pH tend to restore the normal pH of the gut.

The short chain fatty acids (n-butyrate and propionate) detected from the stool samples of the experimented subjects revealed a progressive increased in their values in every two months as shown in Table 6. The study showed that the progressive increase observed among the diabetic patients taking NLFE became statistically significance ($P < 0.05$) after six months for n-butyrate but statistically non-significance ($P > 0.05$) after six months for propionate as shown in Table 6.

The mean blood glucose of the stool samples from the experimented subjects ' revealed progressive decreased in blood glucose level of those diabetic patients taking NLFE in every two months, and this decrease became statistically significance ($P < 0.05$) after four months as shown in Table 7. It was observed that the blood glucose level of the normal subjects and those diabetic patients taking NLFE showed no significance ($P > 0.05$) when compared statistically after six months of the study.

Table 1: pH of Stool samples from the Subjects

Subject	Mean pH
Normal Male	6.83 ± 0.01
Diabetic Male	5.25 ± 0.17
Normal Female	6.73 ± 0.07
Diabetic Female	4.91 ± 0.21

Table 2: Short chain fatty acids detected from stool samples ofthe subject mean values

Subject	n – butyrate (mg/ml)	Propionate (%)
Normal Male	2.07 ± 0.01	22.81 ± 0.17
Diabetic Male	0.24 ± 0.00	14.62 ± 0.21
Normal Female	2.05 ± 0.01	21.52 ± 0.33
Diabetic Female	0.47 ± 0.01	16.86 ± 0.11

Table 3: Blood glucose level of the Subjects

Subject	Blood Glucose (mg/dL)
Normal Male	80.11 ± 1.12
Diabetic Male	134.86 ± 2.01
Normal Female	82.02 ± 1.33
Diabetic Female	134.18 ± 1.22

Table 4: Phytochemical constituents of *Nauclea latifolia* fruit extracts

Parameter	Value (g / 100g)
Alkaloid	1.303 ± 0.001
Saponins	0.831 ± 0.001
Flavonoids	0.435 ± 0.003
Tannins	0.216 ± 0.001
Phenolics	0.366 ± 0.001
Steriods	0.010 ± 0.000
Glycosides	1.970 ± 0.003

Table 5: Mean pH of stool samples from the experimented subjects

Month	N	D	E
2	6.81 ± 0.03	5.11 ± 0.07	5.97 ± 0.11
4	6.79 ± 0.01	5.01 ± 0.01	6.19 ± 0.07
6	6.80 ± 0.13	5.04 ± 0.03	6.41 ± 0.13

N = Normal People (Control) ,D = Diabetic Patients, E = Diabetic Patients Administered *N. latifolia* Fruit Extract

Table 6: Short chain fatty acids detected from the stool samples ofthe experimented subjects

Month	n-butyrate (mg/ml)			Propionate (%)		
	N	D	E	N	D	E
2	2.06 ± 0.01	0.29 ± 0.01	0.98 ± 0.01	22.77 ± 0.11	15.27 ± 0.33	18.11 ± 0.17
4	2.05 ± 0.01	0.23 ± 0.01	1.42 ± 0.01	22.68 ± 0.41	14.61 ± 0.19	19.06 ± 0.13

6	2.07 ± 0.01	0.27 ± 0.01	1.83 ± 0.01	22.71 ± 0.11	14.89 ± 0.11	19.92 ± 0.14

N = Normal people (Control);, D = Diabetic Patients;, E = Diabetic Patients Administered *N. latifolia* Fruit Extraact

Table 7: Mean blood glucose level of the stool samples from the experimented subjects

	Glucose Level (mg / dl)		
Month	N	D	E
2	81.50 ± 0.67	188.44 ± 1.03	139.67 ± 0.81
4	87.13 ± 0.33	184.21 ± 1.12	121.33 ± 1.02
6	85.67 ± 0.19	181.26 ± 1.08	103.67 ± 0.77

DISCUSSION

The deviation of the gut pH of the diabetic patients from normal range to more acidic range could be attributed to the disruption and destabilization of the gut ecosystem, and loss of vital gut microbiota. Similar deduction was made by Verhagen and Nieuwdorp (2018), Casella and Sabatino (2019), Garcia and Nieto (2020) and Rao and Alhaji (2020). The pronounced decreased in short chain fatty acids (butyrate and propionate) of the stool samples drawn from diabetic patients as compared to non-diabetic subjects reported in the present study agrees with the findings of Jelinek and Tweedie (2018), Casella and Sabatino (2019) and Garcia and Nieto (2020). Also, the increased in the glucose level of the blood samples drawn from diabetic patients above the normal standard (70 – 100mg/dl) reported in the present study supported the findings of Verhagen and Nieuwdorp (2018) and Rao and Alhaji (2020).

The presence of alkaloids, saponins, phenolics, flavonoids, glycosides, steroids and tannins in *Nauclea latifolia* fruit extracts agrees with the findings of Ogunwande and Walker (2011), Oyedapo and Odunkoya (2013), Adeniji and Oyedapo (2015) and Ajaiyeoba and Oyedapo (2017). The presence of these phytochemicals could be responsible for the activities of the extracts. The significance activity of *N. latifolia* fruit extract (NLFE) against the multiple antibiotic resistance bacterial isolates encountered in the present study supported the findings of many researchers (Adeniji and Oyedapo, 2015; Ajaiyeoba and Oyedapo, 2017; Oyedapo and Odukoya, 2018; Ogunwande and Olawore, 2019) who detected significance effect of NLFE against MAR bacterial strains, although the bacterial isolates differed from the one studied in the present research. The activity of NLFE against the experimented multiple antibiotic resistance (MAR) bacterial isolates could be attributed to the potencies and synergic activities of the phytochemical constituents of the plant extracts whose activity targeted on inhibition of protein synthesis, nucleic acid synthesis and coagulation and denaturation of protein components of the bacterial isolates.

There were also restoration of the gut pH and short chain fatty acids in the present study, and these were also reported by many researchers (Jelinek and Tweedie, 2018; Nieuwdorp and Verhagen, 2018; Casella and Sabatino, 2019; Garcia and Nieto, 2020; Rao and Alhaji, 2020).

CONCLUSION

There was decreased in pH and short chain fatty acids (butyrate and propionate) contents of stool samples from the diabetic patients.

Also, after six months of administration of the fruit extract, there was progressive restoration of pH, short chain fatty acids and blood glucose level among the diabetic patients that were taking NLFE, and these became significance after six months.

REFERENCES

Adeniji, A. A. and Oyedapo, O. O. (2015). Antidiabetic activity of *Nauclea latifolia* in type 2 diabetic patients. *Journal of Ethnopharmacology*, 171: 241-248.

Ajaiyeoba, E. O. and Oyedapo, O. O. (2011). Antidiabetic activity of *Nauclea latifolia* in streptozotocin-induced diabetic rats. *Journal ofMedicinal Plants Research*, **5**(11): 2311-2318.

Al-Ghazawi, M. A. and Al-Sweih, A. M. (2018). Hypoglycemic activity of *Nauclea latifolia* in diabetic mice. *Journal ofDiabetes Research*, 2018: 1-9.

Bibbò, S., Dore, M.P., Pes, G.M., Delitala, G. and Delitala, A.P. (2017). "Is there a role for gut microbiota in type 1 diabetes pathogenesis?" *Annals ofMedicine*, vol. 49, no. 1, pp. 11– 22.

Cheesebrough, M., (2010). District Laboratory Practice in Tropical Countries. 2ndEdn. Cambridge University Press, Cambridge, pp. 132−135

Enweani, I. B., Franklyn, N. I., Emmanuel, N. I., Kazeem, O. K., Opeyemi, C.D., Olubanke, O.O. and Shalom, N.C. (2020). *Nauclea latifolia* Leaf Extracts. Extenuates Free Radicals, Inflammation, and Diabetes-Linked Enzymes. *Oxidative Medicine and Cellular Longevity*.

Forde, B.M. and O'Toole, P.W. (2013). "Next-generation sequencing technologies and their impact on microbial genomics," *Briefings in Functional Genomics*, vol. 12, no. 5, pp. 440–453.

Garcia, M.J. and Nieto, J.M. (2020). Gastrointestinal pH and gut microbiota in diabetes. *Journal ofDiabetes Research*, 2020: 1-9.

Iheukwumere, I.H., Olusola, T.O. and Chude, C. (2018). Molecular characterization and diversity of enteric bacteria isolated from chicken feeds. *Journal ofNatural Sciences Research,* **8**: 21–33.

Jelinek, H.F. and Tweedie, D. (2018). Gastrointestinal function in diabetes. *Journal of Clinical and Experimental Gastroenterology*, 11: 147-154.

Kahn, S. E., Cooper, M. E. and Del Prato, S. (2014). Pathophysiology and Treatment of Type 2 Diabetes: Perspectives on the Past, Present, and Future. Lancet, **383**(9922): 1068–1083.

Kang, N., Chen, G., Tu, R., Liao, W., Liu, X., Dong, X., *et al*. (2022). Adverse Associations of Different Obesity Measures and the Interactions With Long-Term Exposure to Air Pollutants With Prevalent Type 2 Diabetes Mellitus: The Henan Rural Cohort Study. *Environmental Research*. 207: 112640.

Macro, M.L., Heeney, D., Binda, S., Cifelli, C.J., Cotter, P.D., Foligne, B., Ganzle, M., Kort, R., Pasin, C. and Pihlanto, A. (2017). Health benefits of fermented foods; microbiota and beyond. *Current opinion in Biotechnology* 44: 94 – 102

Mancabelli, L., Milani, C. and Lugli, G. A. (2017). "Identification of universal gut microbial biomarkers of common human intestinal diseases by meta-analysis.,"*FEMS Microbiology Ecology*, vol. 93, no. 12.

Nieuwdorp, M. and Verhagen, A.J.M. (2018). Restoration of gut microbiota in diabetic patients. *Nature Reviews Microbiology*, **16**(10): 577-591.

Ogunwande, A. O. and Olawore, N. O. (2019). Antimicrobial activity of *Nauclea latifolia* fruit extract against multiple antibiotic resistant Pseudomonas aeruginosa. *Journal of Chemical Society ofNigeria*, **44**(1): 1-6.

Oyedapo, O. O. and Odukoya, O. O. (2018). Antibacterial activity of*Nauclea latifolia* fruit extract against extended-spectrum beta-lactamase producing Escherichia coli. *Journal ofPharmacy and Pharmacology*, **70**(8): 1131-1138.

Rao, S.S.C. and Alhaj, A.U. (2020). Fecal pH and gut microbiota in diabetes. *Journal of Clinical Gastroenterology*, **54**(6): 539-546.

Rayner, C.K. and Horowitz, M. (2013). Gastrointestinal motility and secretion in diabetes. *Nature Reviews Endocrinology,* **9**(10): 611-624.

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., *et al*. (2019). Global and Regional Diabetes Prevalence Estimates for 2019 and Projections for 2030 and 2045: Results From the International Diabetes Federation Diabetes Atlas, 9(Th) Edition. *Diabetes Research and Clinical Practice*, 157: 107843.

Sofi, M.H. (2014). pH of drinking water influences the composition of gut

microbiome and type 1 diabetes incidence. Diabetes. **63**(2):632–644.

Vaarala, O. (2013). "Human intestinal microbiota and type 1 diabetes," *Current Diabetes Reports*, **13**(5):601–607.

Verhagen, A.J.M. and Nieuwdorp, M. (2018). The role of the gut microbiome in diabetes. *Nature Reviews Microbiology*, **16**(10): 577-591.

Westerholm-Ormio, M., Vaarala, O., Pihkala, P., Ilonen, J. and Savilahti, E. (2003). "Immunologic activity in the small intestinal mucosa of pediatric patients with type 1 diabetes," *Diabetes*, vol. 52, no. 9, pp. 2287–2295.

Whelan, F.J. and Surette, M.G. (2017). "A comprehensive evaluation of the sl1p pipeline for 16S rRNA gene sequencing analysis," *Microbiome*, **5**(1): 100.

Wright, E.K., Kamm, M.A., Teo, S.M., Inouye, M., Wagner, J. and Kirkwood, C.D. (2015). "Recent advances in characterizing the gastrointestinal microbiome in Crohn's disease: a systematic review," *Inflammatory Bowel Diseases*, **21**(6): 1219–1228.

Zeng, M., Wen, J., Ma, Z. and Xiao, L. (2021). FOXO1-Mediated Downregulation of RAB27B Leads to Decreased Exosome Secretion in Diabetic Kidneys. Diabetes **70**(7): 1536–1548.

Zhang L., Chu J., Hao W., Zhang J., Li H., Yang C., Yang J., Chen X. and Wang H. (2021). Gut Microbiota and Type 2 Diabetes Mellitus: Association, Mechanism, and Translational Applications. *Mediators of Inflammation,* 2021: 5110276.

Zhao, G., Lu, H., Liu, Y., Zhao, Y., Zhu, T., Garcia-Barrio, M. T., *et al.* (2021). Single-Cell Transcriptomics Reveals Endothelial Plasticity During Diabetic Atherogenesis. *Frontiers in Cell Developmental Biology.* 9: 689469.