FUNCTIONAL FOOD JOURNAL

Publication of the Functional Foods and Nutraceuticals Association of Nigeria

A Comparative Study on the in vitro Glucose-Lowering and Antioxidant Properties of Methanol Leaf Extracts of Clove Basil (*Ocimum gratissimum*) and Amaranth Globe (*Gongronema latifolium*)

Osebhahiemen Ibukun*, Victor Ayomide Adedeji. Olanrewaju Wale-Sadeeq

Department of Biochemistry, Faculty of Basic Medical Sciences, University of Medical Sciences, P.O. Box 536, Ondo, Ondo State, Nigeria.

Abstract

There is a need to search for safer compounds for the control of diabetes mellitus due to the side effects of available drugs, including oxidative stress, hepatoinflammation, and gastrointestinal issues. This research studied the in vitro glucose-lowering and antioxidant properties of methanol leaf extracts of *Gongronema latifolium* and *Ocimum gratissimum* using standard protocols. From the results, the methanol leaf extract of *Gongronema latifolium* has significantly higher ($p < 0.05$) glucose-lowering activity than the extract of *Ocimum gratissimum*. However, *Ocimum gratissimum* extract showed significantly higher ($p < 0.05$) antioxidant properties than that of *Gongronema latifolium*. In conclusion, both extracts had appreciable glucose-lowering and antioxidant properties and could be useful in the management of oxidative stress and hyperglycemia associated with diabetes mellitus.

Key words: Glucose, Antioxidant, Diabetes mellitus, *Ocimum gratissimum* and *Gongronema latifolium.*

***Corresponding author:**
oojemekele@unimed.edu.ng

INTRODUCTION

Diabetes mellitus is characterized by hyperglycaemia arising from deficiency of insulin (Zheng *et al.*, 2018). This disorder affects individuals all over the world. A large amount of money is spent by individuals in the management of diabetes mellitus using several drugs. However, most of these drugs have several side effects such as hypoglycemia, nausea, abdominal pain, oxidative stress, hepato-inflammation. This has made it necessary to search for safer and cheaper compounds from plants for diabetes management (Tran *et al*., 2020).

Research has shown the relationship between diabetes and oxidative stress. It has been suggested that free radicals play

Received: 25/03/2024
Received in revised form: 19/05/2024
Accepted: 01/06/2024

major roles in the complications of diabetes due to their role in lipid, DNA, and protein damage. Retinopathy, stroke, and neuropathy are some of the complications arising from diabetes that are mediated by oxidative stress. Studies have also shown the action of hyperglycemia in the production of oxidative stress, which could lead to endothelial dysfunction in patients with diabetes (Luc et al., 2019).

Ocimum gratissimum is a herbaceous plant with a very strong aromatic smell. It is popularly known as scent leaf. Traditionally, it has been found to be useful as an alternative agent for the treatment of many diseases including diarrhea, headache, cough and pneumonia (Akara *et al*., 2021). Research has demonstrated the role of *Ocimum gratissimum* as a gastroprotective, antidiarrhoeal, anti-ulcerative, antimicrobial, and hepatoprotective plant (Melo *et al*., 2019; Akara *et al*., 2021).

Gongronema latifolium is an edible plant with several medicinal properties. It has a bitter taste and it is used traditionally to treat stomach ache, hyperglycemia, cough, malaria, hypertension and liver problems (Amrelia, 2022).

As part of the test for the efficacy of medicinal plants for diabetes mellitus, the aim of this study was to comparatively investigate the in vitro glucose-lowering and antioxidant properties of the methanol leaf extract of two medicinal plants, *Ocimum gratissimum* and *Gongronema latifolium*, commonly used in the management of diabetes mellitus.

MATERIALS AND METHODS

Chemicals and Kits

The glucose oxidase kit was obtained from Randox Laboratories Limited, Antrim, United Kingdom. Baker's yeast was obtained from Guangxi Danbaoli Yeast Co., Ltd., China. Dialysis bags (100 cm) were obtained from Linyeyue Laboratory, China. Glucose powder was obtained from Spectrum Chemical, MFG Corp., California, while other chemicals used were purchased from Sigma Chemical Company, U.S.A.

Plants

Fresh leaves of Ocimum gratissimum and Gongronema latifolium were collected from a private farm in Ile-oluji, Ondo State, Nigeria. This was followed by the identification of the fresh leaves by a botanist at the University of Medical Sciences, Ondo State, Nigeria. Herbarium specimens (voucher numbers P.B.T.H. 024 and P.B.T.H. 025) were placed in the Herbarium for *Ocimum gratissimum* and *Gongronema latifolium*, respectively.

METHODS

Preparation of Plant Extract

The leaves of *Ocimum gratissimum* and *Gongronema latifolium* were thoroughly rinsed with water and dried in the air. The dried leaves were crushed by hand. This was followed by soaking in methanol (72 hours). A muslin bag was used to filter it. A rotary evaporator was then used to concentrate the filtrate obtained at 50 ^{o}C.

Calculation of Percentage Yield

Percentage (%) yield = Weight of dry extract (g) / w=Weight of dry leaves (g) × 100

Ocimum gratissimum % yield = 12.77 / 177.8 × 100 = 7.18%

Gongronema latifolium % yield= 10.84 / 100.68 × 100 = 10.77%

Evaluation of *In vitro* Glucose-Lowering Activity of Plant Extracts

The glucose adsorption capacity of the samples was determined by the method of (Ou et al. (2001). The effect of the plant extracts on glucose uptake by yeast cells was determined by the method of Cirillo (1962), and the effect of plant extracts on in-vitro glucose diffusion was determined according to the method stated by (Ahmed et al., 2011).

Evaluation of *In vitro* Antioxidant Activity of the Plant Extracts

Ferric Reducing Antioxidant Power (FRAP) Assay

The Ferric Reducing Antioxidant Power (FRAP) assay was carried out using the method of Benzie and Strain (1996). Total antioxidant capacity (TAC) was measured using the method described by Prieto et al. (1999) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was determined by a method described by Brand-Williams et al. (1995).

Statistical Analysis

All experiments were carried out in triplicate, and the data were analyzed by the student t-test or ANOVA, followed by Tukey's multiple comparisons test where applicable, to check for significant differences. The significance level was set at $p < 0.05$. Graph plotting as well as analysis were carried out using GraphPad Prism 10 software.

RESULTS

Glucose-lowering Property of *Ocimum gratissimum* and *Gongronema latifolium* Leaf Extracts

Glucose Adsorption Capacity

Glucose adsorption by the extracts was proportional to the molar concentration of glucose. Therefore, higher concentration of glucose was adsorbed by the extracts at higher glucose levels. At lower glucose concentration (5mM), there was no significant difference ($p > 0.05$) between the adsorption capacities of *Ocimum gratissimum* and *Gongronema latifolium* However at higher concentrations of glucose, the glucose adsorption capacity of *Gongronema latifolium* extract was significantly higher ($p < 0.05$) than that of *Ocimum gratissimum* extract (Figure 1).

Effect of *Ocimum gratissimum* and *Gongronema latifolium* leaf extracts on glucose transport across yeast cells

The extract of *Gongronema latifolium* had significantly higher ($p < 0.05$) enhancement of glucose uptake activity by yeast cells than *Ocimum gratissimum* extract at all concentrations (Figure 2).

Effect of *Ocimum gratissimum* and *Gongronema latifolium* leaf extracts on *in vitro* glucose diffusion and glucose diffusion retardation index (GDRI)

The rate of glucose diffusion was found to increase with time (from 30 to 60 minutes). It was found that the extracts significantly inhibited the transport of glucose into solution from the dialysis membrane in comparison with the control (Table 1). Glucose diffusion retardation by *Gongronema latifolium* extract was significantly higher than that of *Ocimum gratissimum* at 30 minutes. This was indicated by a higher GDRI value for *Gongronema latifolium* than *Ocimum gratissimum* at 30 minutes.

***In vitro* antioxidant property of *Ocimum gratissimum* and *Gongronema latifolium* leaf extracts**

Figure 3 shows the ferric reducing antioxidant power (FRAP) of the extracts. Result indicated that *Ocimum gratissimum* extract had a significantly higher FRAP than ascorbic acid and *Gongronema latifolium* extract. Furthermore, there was no significant difference between *Gongronema latifolium* extract and ascorbic acid.

Figure 4 shows the total antioxidant capacity of *Ocimum gratissimum* extract had a significantly higher ($p < 0.05$) total antioxidant capacity than that of *Gongronema latifolium* extract.

Figure 5 shows the DPPH radical scavenging activity of *Ocimum gratissimum* and *Gongronema latifolium*, with IC_{50} values shown in table 3. *Ocimum gratissimum* extract had a significantly higher ($p < 0.05$) DPPH

radical scavenging activity than that of *Gongronema latifolium* extract as indicated by IC_{50} values. However, ascorbic acid had a significantly higher DPPH radical scavenging activity than both extracts.

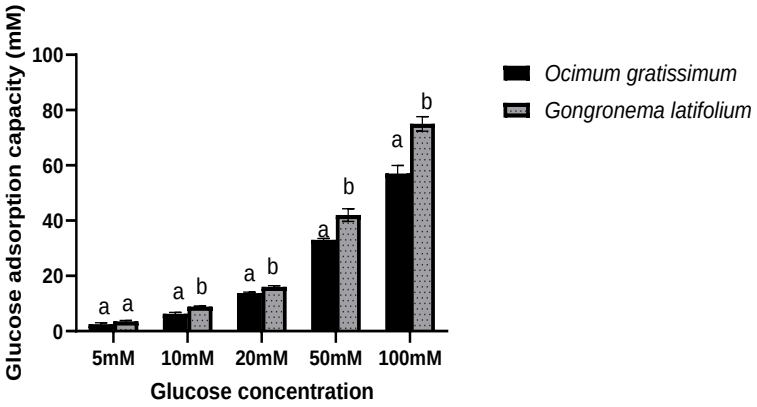

Figure 1: Glucose adsorption capacity of *Ocimum gratissimum* and *Gongronema latifolium* leaf extracts
Results are expressed as Mean ± SEM. Different alphabets on bars for the same glucose concentration indicate that means are significantly different ($p < 0.05$).

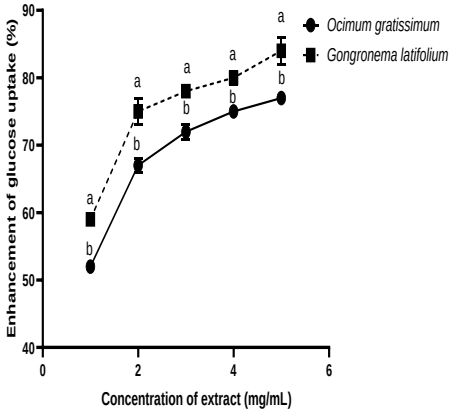

Figure 2: Effect of *Ocimum gratissimum* and *Gongronema latifolium* extracts on the uptake of glucose by yeast cells. Results are expressed as Mean ± SEM. Different alphabets on lines for the same concentration indicate that means are significantly different ($p < 0.05$).

Table 1: Effect of Ocimum gratissimum and Gongronema latifolium leaf extracts on in vitro glucose diffusion

Sample	**GDRI**	
	30mins	**60mins**
Ocimum gratissimum	^{a}60.31±0.76	^{a}61.81±2.01
Gongronema latifolium	^{b}68.85±1.26	^{b}21.78±3.69

Results are expressed as Mean ± SEM. Different alphabets down the column indicate that means are significantly different ($p < 0.05$).

Table 2: Effect of *Ocimum gratissimum* and *Gongronema latifolium* leaf extracts on GDRI

Sample	**Glucose content in dialysate (mmol/L)**	
	30mins	**60mins**
Control	^{a}3.98±0.01	^{a}5.97±0.01
Ocimum gratissimum	^{b}1.58±0.03	^{b}2.28±0.12
Gongronema latifolium	^{c}1.24±0.05	^{c}4.67±0.22

Results are expressed as Mean ± SEM. Different alphabets down the column indicate that means are significantly different ($p < 0.05$).

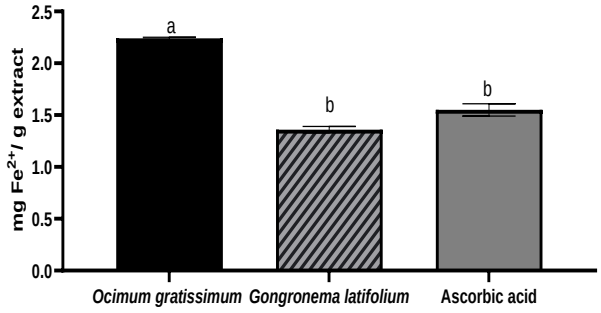

Figure 3: Ferric reducing antioxidant power of *Ocimum gratissimum* and *Gongronema latifolium* leaf extracts
Results are expressed as Mean±SEM. Different alphabets bars indicate that means are significantly different ($p < 0.05$).

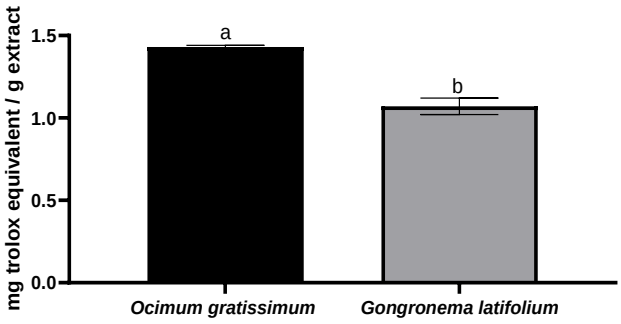

Figure 4: Total antioxidant capacity of *Ocimum gratissimum* and *Gongronema latifolium* leaf extracts
Results are expressed as Mean±SEM. Different alphabets bars indicate that means are significantly different ($p < 0.05$).

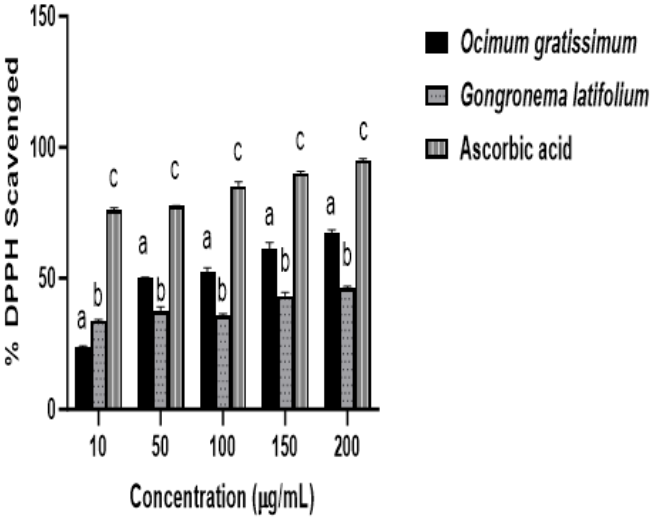

Figure 5 : DPPH radical scavenging activity of Ocimum gratissimum and Gongronema latifolium leaf extracts
Results are expressed as Mean±SEM. Different alphabets bars (for same concentration) indicate that means are significantly different (p < 0.05).

Table 3: DPPH IC_{50} values of *Ocimum gratissimum* and *Gongronema latifolium* leaf extracts

Extract	IC_{50} (µg/mL)
Ocimum gratissimum	^{a}97.49±2.39
Gongronema latifolium	^{b}267.55±4.90
Ascorbic acid	^{c}3.3±0.05

Results are expressed as Mean±SEM. Different alphabets indicate that means are significantly different ($p < 0.05$).

DISCUSSION

This study evaluated the *in vitro* glucose-lowering and antioxidant properties of methanol leaf extracts of *Ocimum gratissimum* and *Gongronema latifolium*. The glucose adsorption capacity by *Gongronema latifolium* was significantly higher ($p < 0.05$) than that of *Ocimum gratissimum* at higher glucose levels. Findings from this test implies that the extracts can adsorb glucose, may lower the amount of glucose available for transportation through the intestinal lumen, and as a result, reducing postprandial hyperglycemia (Mangesh *et al.*, 2018). The adsorption activity has been attributed to dietary fiber in some studies (Rehman *et al*., 2018). However, it could result from other metabolites such as phenolic compounds as it was more dependent on the content of these compounds in the extracts. Similar observations have been reported by Tan *et al*. (2004) who studied glucose adsorption capacity of coconut fibers.

Glucose transport across the yeast cell membrane is a useful method for determining the in vitro hypoglycemic properties of several compounds (Cherbal et al., 2017). This study showed that both extracts promoted glucose

transport across yeast cells. However, *Gongronema latifolium* extract showed a significantly higher enhancement of glucose uptake activity than *Ocimum gratissimum* extract. The findings from this study imply that methanol leaf extract of *Gongronema latifolium* enhanced glucose uptake more effectively than that of *Ocimum gratissimum*, and could enhance a better blood glucose flow in the body. This finding might be attributed to the degree of solubility of various compounds from plant extracts in the solvent of extraction (Rehman *et al*., 2018).

The retardation of glucose diffusion across the dialysis bag by the extracts of *Ocimum gratissimum* and *Gongronema latifolium* was also carried out. It was shown that both extracts significantly inhibited the diffusion of glucose across the dialysis membrane. However, the glucose diffusion retardation by *Gongronema latifolium* extract was significantly higher than *Ocimum gratissimum* at 30 minutes. Retardation of glucose diffusion was determined by GDRI which shows the extent to which a compound can retard diffusion of glucose in comparison with a control. Glucose dialysis retardation is useful in predicting the effect of the extract on the delay of glucose absorption in the gastrointestinal tract. The extracts may slow down the rate of glucose transport to the small intestinal epithelium, thus decreasing postprandial hyperglycemia.

Antioxidants are substances that cause a barrier from free radical damage resulting in delay of oxidation. Antioxidants scavenge free radicals by donating one of their own electrons to free radicals (which have unpaired electron) and this helps in the prevention of cellular damage and resultant diseases. (Martemucci *et al*., 2022)

FRAP assay is based on the reduction of Fe^{3+} to Fe^{2+} by antioxidants (Isildak *et al.*, 2023). This study showed that *Ocimum gratissimum* extract had a significantly higher ferric reducing antioxidant capacity than *Gongronema latifolium* extract. Total antioxidant capacity (phosphomolybdenum assay) measures the degree to which an antioxidant causes a reduction of Mo (VI) to Mo (V) (Gupta *et al*., 2020). In this study, *Ocimum gratissimum* showed a higher total antioxidant capacity than *Gongronema latifolium*. DPPH assay measures the ability of an antioxidant to scavenge DPPH radical (Baliyan *et al.*, 2022). Methanol leaf extract of *Ocimum gratissimum* scavenged DPPH radical in a higher degree than *Gongronema latifolium* extract. Therefore, all these findings suggest that the methanol leaf extract of *Ocimum gratissimum* has greater antioxidant capacity than that of *Gongronema latifolium*.

The antioxidant properties of *Ocimum gratissimum* and *Gongronema latifolium* extracts could be attributed to the presence of phytochemicals in the plants. The antioxidant capacity of plants has been suggested to be a combination of the roles played by the different groups of phytochemicals rather than any one group (Akinrinde et al., 2018). Therefore, the higher the concentration of phytochemicals in a plant, probably the higher the antioxidant activity of the plant (Ibukun and Oluwadare, 2021). Amelia et al. (2022) have revealed that saponins, flavonoids, alkaloids, tannins, and phenols are found in the leaves of *Gongronema latifolium*. Research has revealed that polyphenols, eugenols, thymols, and flavonoids are abundant in the leaves of *Ocimum gratissimum* (Talabi and Makanjuol, 2017; Olamilosoye et al., 2018).

CONCLUSION

This study has proven that the methanol leaf extract of *Gongronema latifolium* has better anti-hyperglycemic activity than the methanol leaf extract *of Ocimum gratissimum*. However, the methanol leaf

extract of *Ocimum gratissimum* showed greater antioxidant capacity than that of *Gongronema latifolium*. Both extracts of *Ocimum gratissimum* and *Gongronema latifolium* could be said to have an appreciable therapeutic effect on diabetes and oxidative stress. However, further studies (in vivo) are needed to further validate these findings.

FINANCIAL SUPPORT
None

CONFLICT OF INTEREST
The authors haven no conflcts of interest to declare

ACKNOWLEDGEMENT
The authors acknowledge the members of staff of Biochemistry department, Faculty of Basic Medical Sciences, University of Medical Sciences, Ondo.

AUTHOR CONTRIBUTION
OI contributed to conceptualization methodology, data analysis, supervision, validation, visualization, review and editing of the manuscript.
VA and OW contributed to data acquisition, methodology, resources and writing of the original draft of the manuscript.

REFERENCES

Akara, E., U., Okezie, E., Ude, V., C., Uche-Ikonne, C., Eke, G. and Ugbogu, A., E., (2021). *Ocimum gratissimum* leaf extract ameliorates phenylhydrazine-induced anaemia and toxicity in Wistar rats. *Drug Metab. Pers. Ther*, 42(5): 344-352

Akinrinde, A., S., Afolayan, A., J., M. and Bradley, G. (2018). Phytochemical composition and antioxidant activities of Dianthus thunbergii Hooper and Hypoxis argentea Harv ex Baker: plants used for the management of diabetes mellitus in Eastern Cape, South Africa. *Pharmacogn Mag*, 14(54):195-202.

Amrelia, M. (2022). Nutritive and medicinal value of Gongronema latifolium. *J. Nat. Prod. Dis.* 1(2):22-36.

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R.P. and Chang, C.M. (2022). Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. *Molecules*, 27(4):13-26.

Benzie, I. F. and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, 239(1): 70-76.

Brand-Williams, W., Cuvelier, M., E. and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology*, 28(1):25-30.

Cherbal, A., Kebieche, M., Yilmaz, E.M., Aydoğmuş, Z., Benzaouia, L., Benguessoum, M., Benkedidah, M. and Madani, K. (2017). Antidiabetic and hypolipidemic activities of Algerian Pistachia lentiscus L. leaves extract in alloxan-induced diabetic rats. *South African Journal of Botany*, 108: 157-162.

Cirillo, V. P. (1962). Mechanism of glucose transport across the yeast cell membrane. *J. bacteriol*, 84(3): 485-491.

Gupta, S., Finelli, R., Agarwal, A. and Henkel, R. (2020). Total antioxidant capacity—Relevance, methods and clinical implications. *Andrologia*, 53:1-8.

Ibukun, O. and Oluwadare, E., E. (2021). In vitro Antioxidant Property and Acute Toxicity Study of Methanol Extract of Leaves of Zingiber officinale and Curcuma longa. *Free Radicals and Antioxidants*, 11(2): 42-5.

Isildak, O., Yildiz, I., Genç, N., Sabanci, D. and Işildak, I. (2023). New potentiometric PVC membrane electrode for Ferric Reduction Antioxidant Power assay. *Food Chem,* 423:136-148.

Luc, K., Schramm-Luc A., Guzik T.J. and Mikolajczyk T.P. (2019). Oxidative stress and inflammatory markers in prediabetes and diabetes. *J. Physiol. Pharmacol.* 70:809-824.

Mangesh, B., Somnath, B., Dheeraj, R., Ganesh, W. and Sachin, T. (2018). Studies on glucose adsorption capacity of some indigenous plants. *Global journal of pharmacy and pharmaceutical sciences*, 5(1): 2250-2573.

Martemucci, G., Costagliola, C., Mariano, M., D'andrea, L, Napolitano, P. and D'Alessandro, A.G. (2022). Free Radical Properties, Source and Targets, Antioxidant Consumption and Health. *Oxygen*, 2:48-78.

Melo, R.S., Azevedo, A.M.A., Pereira, A.M.G., Rocha, R.R., Cavalcante, R.M.B.,Matos, M.N.C., Lopes, P.H.R., Gomes, G.A., Rodrigues, T.H.S., Dos Santos, H.S.,Ponte, I.L., Costa, R.A., Brito, G.S., Júnior, F.E.A.C. and Carneiro, V.A. (2019). Chemical composition and antimicrobial effectiveness of *Ocimum gratissimum* L. Essential oil against multidrug-resistant isolates of Staphylococcus aureus and Escherichia coli. *Molecules*, 3864, 2-17.

Olamilosoye, K.P., Akomolafe, R.O., Akinsomisoye O.S., Adefisayo M.A. and Alabi Q.K. (2018). The aqueous extract of *Ocimum gratissimum* leaves ameliorates acetic acid-induced colitis via improving antioxidant status and hematological parameters in male Wistar rats. *Egypt. J. Basic Appl. Sci,* 5:220-227.

Ou, K., Kwok, C., Li, Y. and Fu, L. (2001). *In vitro* study of possible role of dietary fiber in lowering postprandial serum glucose. *Journal of Agricultural and Food Chemistry*, 49(2): 1026–1029.

Prieto, P., Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry*, 269(2): 337-341.

Rehman, G., Hamayun, M., Iqbal, A., Islam, S. U., Arshad, S., Zaman, K., Ahmad, A., Shehzad, A., Hussain, A. and Lee, I. (2018). In Vitro Antidiabetic Effects and Antioxidant Potential of Cassia nemophila Pods.*J. Bio. Innov*, 5(1): 24-30.

Talabi, J.Y. and Makanjuola S. (2017). Proximate, Phytochemical, and i*n vitro* Antimicrobial Properties of Dried Leaves from *Ocimum gratissimum*. *Prev. Nutr. Food Sci*, 22:191-194. doi: 10.3746/pnf.2017.22.3.191.

Tan, Y., Li, S., Li, C. and Liu, S. (2023). Glucose adsorption and α-amylase activity inhibition mechanism of insoluble dietary fiber: Comparison of structural and microrheological properties of three different modified coconut residue fibers. *Food Chem*, 418: 135-146.

Tran, N., Pham, B. and Le, L. (2020). Bioactive Compounds in Anti-Diabetic Plants: From Herbal Medicine to Modern Drug Discovery. *Biology (Basel),* 9(9): 252-266.

Zheng, Y, Ley, S.H. and Hu, F.B. (2018). Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol*. 14(2):88-98.

Cite as: Ibukun O., Adedeji. V.A. & Wale-Sadeeq O. (2024).A Comparative Study on the in vitro Glucose-Lowering and Antioxidant properties of Methanol Leaf Extracts of Ocimum gratissimum and Gongronema latifolium Funct Food J 5(1):44-52.

.