FULafia Journal of Science & Technology
ISSN (print) 2449-0954 \ ISSN (online) 2636-4972
https://lafiascijournals.org.ng/index.php/fjst
Sponsored By TETFUND TERTIARY EDUCATION TRUST FUND
Manuscript => Received: June, 2025; Accepted: Sept, 2025; Published: Sept., 2025 https://doi.org/10.62050/fjst2025.v9n2.468

IN VITRO ANTIOXIDANT AND ANTIHYPERGLYCEMIA PROPERTIES OF VERBENONE ENHANCED WITH SELECTED SOLUBILIZING COMPOUNDS

Oluremi A. Saliu[1], Blessing Edogbo[1], Habibu Tijjani[1]*, Ayodeji O. Idowu[1], Shehu Usman Adamu[2], Suwaiba Nasir[1], Salima Adamu Sada[1], Moses Dele Adams[3] and Noel Biodun Saliu[4]

[1]*Department of Environmental Health Science, National Open University of Nigeria, Abuja, Nigeria*
[2]*Department of Public Health, National Open University of Nigeria, Abuja, Nigeria*
[3]*Clinical Biochemistry, Phytopharmacology and Biochemical Toxicology Research Laboratory (CBPBT-RL), Department of Biochemistry, Baze University, Abuja, Nigeria*
[4]*National University of Science and Technology, Abuja, Nigeria*
*Corresponding email: hatijjani@noun.edu.ng; haatscific@gmail.com

ABSTRACT

The pharmacological activities of verbenone had been reported in several diseased states. This study evaluated the *in vitro* antioxidant and antihyperglycemia properties of verbenone through linkage with selected solubilizing compounds. Oral Glucose Tolerance Test (OGTT) was conducted in non-diabetic mice overloaded with 2 g/kg bwt. glucose solution. *In vitro* α amylase and α glucosidase inhibitory activities were evaluated for verbenone and selected solubilizing compounds. 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2) and hydroxyl (OH) radical scavenging activity were evaluated for verbenone and selected solubilizing compounds using references procedures. In OGTT assay, verbenone, lysine, cyclodextrin and metformin significantly ($p<0.05$) lowered glucose concentration compared with the control from 30 - 120 minutes. Lysine and verb-lysine (12.5 µg/mL) steadily raised the percentage α amylase inhibitory activity, while at the same concentration significantly *($p<0.05$)* increased amylase activity from 70 to 95%. Acarbose and all concentrations of compound examined (12.5 - 200 µg/mL) significantly *($p<0.05$)* increased the percentage inhibitory activity of α glucosidase. All concentrations of verbenone significantly increased the percentage scavenging activities of DPPH, H_2O_2 and OH. Lysine, cyclodextrin verb-lysine showed a significantly *($p<0.05$)* high hydroxyl scavenging while verb-cyclodextrin and acarbose displayed a lower hydroxyl scavenging. The results indicated that verbenone and its linked solubilizing compounds exhibited *in vitro* antihyperglycemic properties in glucose loaded mice, increased inhibitory activities of α amylase and α glucosidase as well as increased *in vitro* antioxidant scavenging activities.

Keywords: Verbenone, antioxidant, antihyperglycemia, α amylase, α glucosidase, solubilizing compounds

INTRODUCTION

Glycaemia is a measure of the quantity of blood glucose in the body. The amount of circulating glucose in the body is influenced by dietary ingestion of carbohydrate, extent of exercise, sedentary pattern, gluconeogenetic pathway, action of enzyme inhibitors, enzymatic control and hormonal fluctuations (Gudise *et al*., 2021). The maintenance and control of glucose and glycaemic index is achieved by interplay between carbohydrate hydrolyzing enzymes like amylase and glucosidase as well as glucose controlling hormones namely: insulin and glucagon (Asmat *et al*., 2016). Uncontrolled glycaemia index presents with hyperglycaemia which involves elevated blood glucose level (Asmat *et al*., 2016).

Hyperglycaemia predisposes to diabetes mellitus. Diabetes mellitus is divided into two main types: Type 1 Diabetes Mellitus (T1DM, Insulin-dependent diabetes with 5–10% prevalence) and Type 2 Diabetes Mellitus (T2DM, Non-insulin Dependent Diabetes Mellitus with a prevalence of 90–95%). Diabetes mellitus presents with chronic hyperglycemia, oxidative stress and dyslipidemia which are leads to insulin resistance and mild damage to pancreatic beta cells, causing about 90% of diabetic morbidity and mortality (IDF, 2021). Hyperglycaemia causes oxidative stress by reducing insulin secretion as well as initiating cell and tissue damage, by NADPH-oxidase activation, hexosamine pathway, glucose autoxidation, advanced glycation end products (AGEs) and reactive oxygen species (ROS) production (Bhatti *et al*., 2022).

Oxidative stress is a condition that results in imbalance in the capacity of the biological system to produce free radicals, reactive oxygen species (ROS) and its response to salvage them. Oxidative stress occurs in hyperglycaemia and diabetes via altered glucose metabolism, oxidative damage to pancreatic beta cells, endothelial dysfunction and enzymatic sources (xanthine oxidase, NOS and NADPH oxidase). Oxidative stress can therefore lead to a number of abnormalities and complications including impaired insulin secretion, cellular damage and insulin resistance (Binjawhar *et al*., 2023). Oxidative stress is measured by determination of levels of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), thiobarbituric acid reactive substances (TBARS), nitric oxide (NO), hydroxyl radical (OH) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) among others.

Antioxidants are compounds that possess the ability to delay unwanted peroxidation and prevent oxidative damage to tissues and biological molecules. Elevated levels of free radicals in the body have the ability to damage major biochemical components of cells and tissues (Ado *et al*., 2024). Several compounds have been shown to possess such antioxidants properties. The mode of action of antioxidants may be via enhancement of activity and expression of antioxidant enzymes such as glutathion peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) and decrease in cellular level of free radicals either by inhibiting the activities and expressions of free radical generating enzymes such as NADPH oxidase and xanthine oxidase (XO) (Attama *et al*., 2023).

Hyperglycaemia and oxidative stress have become a global burden relative to its ever increasing morbidity and mortality index despite treatment regimens through antihyperglycaemic and antioxidant drugs which comes with side effects like hypoglycemia, cardiovascular diseases, nausea, oxidative stress body weight gain as well as allergy, and discomfort respectively (Kokil *et al*., 2015). These side effects call for alternative and complementary options in natural products.

Verbenone (4,6,6-trimethylbicyclohept-3-en-2-one) is a liquid ketone-based carbobicyclic natural compound and found freely occurring in plants. Verbenone, which was first reported from *Dendroctonus ponderosae, are* classified as terpene*is and* obtained by auto-oxidation of α-pinene. Verbenone is reported to possess cancer healing property (Song *et al*., 2005), *in vivo* attenuation of oxidative stress in diabetic mice (Tijjani *et al*., 2023; Ado *et al*., 2024), haemolytic, anti-inflammatory, bronchodilating abilities (Rappaport *et al*., 2001), *in vitro* antidiabetic and *in vivo* hyperlipidemic properties in NAD-STZ-induced diabetic mice (Tijjani *et al*., 2022), anti-ischemic property (Ju *et al*., 2013), *in vitro* and *in vivo* breast cancer healing activity (Mander *et al*., 2019; Yang & Hu, 2023). Despite these verbenone related investigations, its poor solubility hinders its maximum biological usage (Tijjani, 2022). Thus, this study evaluated the antioxidant and antihyperglycemia properties of verbenone enhanced with selected solubilizing compounds.

MATERIALS AND METHODS

Drugs and Chemicals

Potato starch and maltose were procured from SD fine chemicals, Mumbai. α-amylase, α-glucosidase, and sodium potassium tartarate tetrahydrate were obtained from Gentex Pharmaceuticals and C. Huitai, Thailand. Acarbose was obtained from Orchid Healthcare, Chennai, India. Glucose kit was procured from Agappe Diagnostic, Switzerland. All other chemicals used in the study were of analytical grade.

Ethical Clearance

Ethical clearance for the study was obtained from the Ethical Review Committee, National Open University of Nigeria (NOUN) with the reference number ETC/2024/NOUN/04/011.

Preparation of Various Concentrations of Verbenone Enhanced Solubilizing Compounds

Concentrations of 12.5, 25, 50, 100 and 200 µg/mL each for verbenone, lysine, vitamin C, verb-lysine, verb-cyclodextrin and cyclodextrin were prepared and used for the study.

Oral glucose tolerance test (OGTT)

Thirty five (35) non-diabetic mice were randomly distributed into seven (7) groups after checking their baseline glucose levels using a glucometer (Accu-Chek Active). A standard oral dose of glucose (2 g/kg bwt.) was administered orally to each mouse after overnight fasting for 12 hours. The blood glucose levels were monitored at regular intervals to evaluate the glucose tolerance ability following treatment with water (control), metformin, verbenone, lysine, cyclodextrin, verb-lysine and verb-cyclodextrin at 12.5 mg/kg bwt. Blood glucose levels were measured from tail veins after 30 minutes, 1, 2 and 4 hours of glucose administration.

Determination of *in vitro* α amylase and α-glucosidase inhibitory activities

α-amylase and α-glucosidase inhibition assays were carried out using the methods described by Andrade-Cetto *et al*. (2008) and Kuppusamy *et al*. (2011) respectively at the concentrations of 12.5 - 200 µg/mL using acarbose as reference drug.

Determination of *In vitro* Antioxidant Activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity

A known amount (500 µL) of 0.11 mM methanolic DPPH was added to 500 µL of different concentrations (12.5 - 200 µg/mL) of verbenone, lysine, cyclodextrin, verb-lysine and verb-cyclodextrin were incubated at room temperature in the dark for 10 minutes (McCune and Johns, 2002). The absorbance of the blank (A_b) and samples (A_s) was measured at 517 nm. % DPPH scavenging activity was calculated using the formula:

$$\%\ DPPH\ scavenging\ activity = (1 - \frac{Abssample}{Absblank}) \times 100$$

Hydrogen peroxide (H_2O_2) scavenging assay

Hydrogen peroxide (H_2O_2) scavenging activity was determined according to the procedure described by Ruch *et al*. (1989). Briefly, 3.4 mL of varying concentration (12.5 - 200 µg/mL) of verbenone, lysine, cyclodextrin, verb-lysine and verb-cyclodextrin in phosphate buffer saline (pH 7.4) were mixed with 0.6 mL of 40 mM H_2O_2. The absorbance was read at 230 nm after 10 minutes of incubation at room temperature.

Hydroxyl (OH^-) scavenging activity

Hydroxyl radical (OH^-) scavenging activity of the extract was measured as described by Smirnoff and Cumbes (1989). Briefly, 2 mL of verbenone, lysine, cyclodextrin, verb-lysine and verb-cyclodextrin at concentrations of 12.5 - 200 µg/mL, 0.6 mL of 8 mM ferrous sulphate, 0.5 mL of 20 mM H_2O_2, and 2 mL of 2 mM salicylic acid were mixed and incubated at 37°C for 30 minutes. Then, 0.9 mL of distilled water was

added to each vial. The final solution was centrifuged at 4472 ×g for 10 minutes after which the absorbance was read at 510 nm.

Statistical Analysis

Experimental data were expressed as mean ± standard error of mean (SEM) and were subjected to One Way Analysis of Variance (ANOVA) followed by Dunnett's test. Significance was considered at $p<0.05$. Graphs were plotted using GraphPad Prism 6 software (GraphPad Software, California, USA).

RESULTS AND DISCUSSION

Verbenone, lysine and cyclodextrin significantly *($p<0.05$)* lowered glucose concentration from 30 - 120 minutes in a manner comparable with that of the reference antihyperglycaemic drug, metformin (Figure 1).

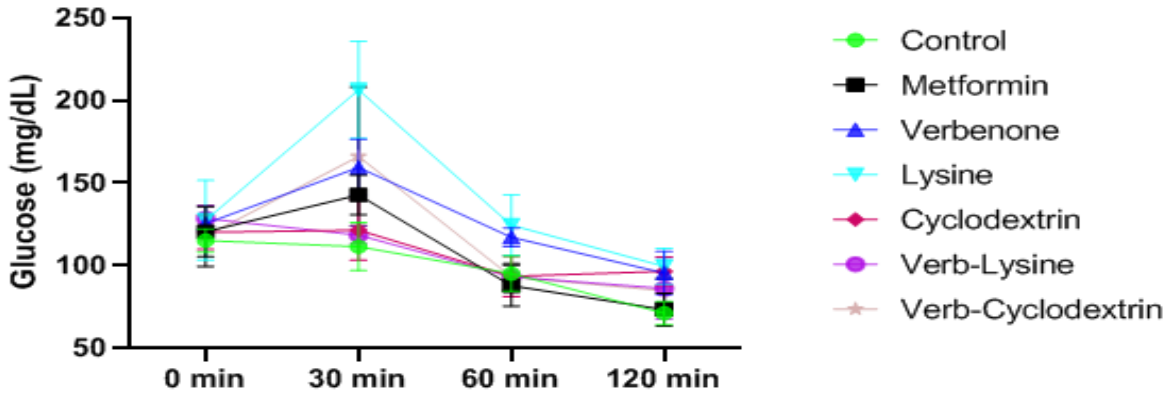

Figure 1: Antihyperglycemic effects of verbenone enhanced with selected solubilizing agents
Values are mean ± SEM

Across the row, the percentage blood glucose level of the control was significantly *($p<0.05$)* reduced from 115.00 (Baseline) to 44.00 (at 120 minutes) with an average percentage of 40.74. Similarly, the percentage blood glucose level of the verbenone was significantly *($p<0.05$)* reduced from 125.33 (Baseline) to 30.00 (at 120 minutes) with an average percentage of 23.08. A similar pattern of percentage glucose reduction was observed across the row for lysine, cyclodextrin, verb-lysine and verb-cyclodextrin. Across the row, the pattern of percentage glucose reduction of cyclodextrin and verb-lysine was comparable *($p>0.05$)* with that of the reference antihyperglycaemic drug, metformin (Table 1).

Table 1: Effects of verbenone enhanced with selected solubilizing agents on percentage blood glucose levels

Groups	Baseline (mg/dL)	30 Min. (%)	60 Min. (%)	120 Min. (%)	Average (%)
Control	115.00	3.67 (↓3.40)	20.00 (↓18.52)	44.00 (↓40.74)	↓40.74
Metformin	120.33	22.33 (↑23.02)	32.67 (↓33.68)	47.00 (↓48.45)	↓48.45
Verbenone	125.33	34.00 (↑26.15)	8.33 (↓6.41)	30.00 (↓23.08)	↓23.08
Lysine	127.33	45.67 (↑26.71)	3.00 (↓1.75)	28.00 (↓16.37)	↓16.37
Cyclodextrin	120.00	1.33 (↑1.09)	26.67 (↓21.86)	23.67 (↓19.40)	↓19.40
Verb-Lysine	128.33	10.00 (↓6.99)	35.33 (↓24.71)	42.33 (↓29.60)	↓29.60
Verb-Cyclodextrin	108.50	36.50 (↑45.06)	19.50 (↓24.07)	27.50 (↓33.95)	↓33.95

Values are mean, ↑ = increase, ↓ = decrease

The 12.5 µg/mL concentration of verbenone significantly *($p<0.05$)* increased the percentage inhibitory activity of α-amylase from 70 to 95%. All examined concentrations (12.5, 25, 50, 100 and 200 µg/mL) of lysine, cyclodextrin, verb-lysine, verb-cyclodextrin and acarbose did not significantly *($p>0.05$)* alter the percentage inhibitory activity of α amylase (Figure 2).

All examined concentrations (12.5, 25, 50, 100 and 200 µg/mL) of verbenone, lysine, cyclodextrin, verb-lysine and verb-cyclodextrin significantly *($p<0.05$)* increased the percentage inhibitory activity of α glucosidase in a manner compared well with acarbose, the reference antihyperglycaemic drug (Figure 3).

Lysine, cyclodextrin, verb-lysine, verb-cyclodextrin and acarbose showed high inhibition against α amylase activity with lowered IC_{50} values of 6.94±0.21, 6.70±0.26, 7.17±0.20, 6.71±0.05 and 6.55±0.05 µg/Ml, respectively (Table 2). Lysine, cyclodextrin, verb-cyclodextrin showed highest inhibition against α glucosidase activity with the lowest IC_{50} value of 11.47±1.93, 12.20±1.14 and 11.90±1.89 µg/mL (Table 2). However, Verb-Lysine displayed the least inhibition against α glucosidase with the highest IC_{50} value of 22.79±7.41 µg/mL. The α glucosidase IC_{50} value of acarbose is 18.59±3.55 µg/mL (Table 2).

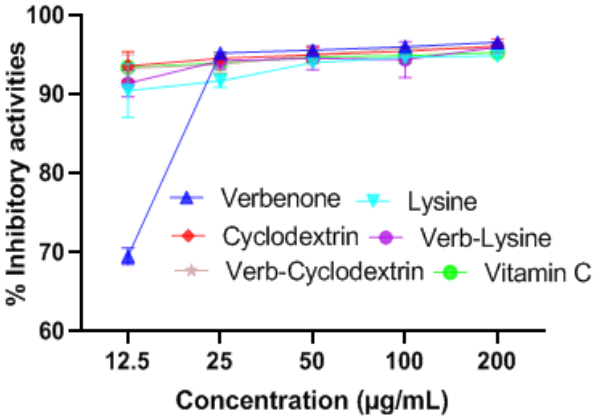

Figure 2: *In vitro* inhibitory activity of α amylase by verbenone enhanced with selected solubilizing compounds
Values are mean ± SEM

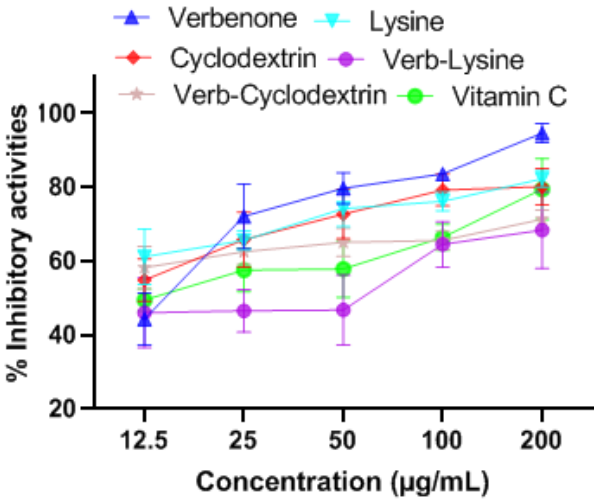

Figure 3: *In vitro* inhibitory activity of α glucosidase by verbenone enhanced with selected solubilizing compounds
Values are mean ± SEM

Table 2: IC_{50} values of inhibition of α amylase and α glucosidase activities by verbenone enhanced with selected solubilizing compounds

Groups	IC_{50} values (µg/mL)	
	α Amylase	α Glucosidase
Verbenone	9.02±0.27[a]	16.10±3.17[a]
Lysine	6.94±0.21[b]	11.47±1.93[b]
Cyclodextrin	6.70±0.26[b]	12.20±1.14[b]
Verb-Lysine	7.17±0.20[b]	22.79±7.41[b]
Verb-Cyclodextrin	6.71±0.05[b]	11.90±1.89[b]
Acarbose	6.55±0.05[b]	18.59±3.55[b]

Values are mean ± SEM. Significant difference are indicated using different superscript at p<0.05. IC_{50} =50% Inhibitory Concentration

All concentrations of lysine, cyclodextrin and verb-cyclodextrin significantly *(p<0.05)* increased the percentage *in vitro* DPPH scavenging activity in a manner comparable with verbenone and vitamin C. However, verb-lysine which the increased the percentage *in vitro* DPPH scavenging activity at 12.5 - 50 µg/mL, reduced it at 100 and 200 µg/mL (Figure 4).
All concentrations (12.5, 25, 50, 100 and 200 µg/mL) of verbenone substantively *(p<0.05)* increased the percentage *in vitro* H_2O_2 scavenging activity. However, all examined concentrations did not significantly *(p>0.05)* alter the percentage *in vitro* H_2O_2 scavenging activity of lysine, cyclodextrin, verb-lysine, verb-cyclodextrin and vitamin C (Figure 5).
All concentrations of verbenone, verb-cyclodextrin, verb-lysine, lysine and cyclodextrin significantly *(p<0.05)* increased the *in vitro* percentage. ·OH scavenging activity in a similar pattern of increase observed in Verbenone and Vitamin C (reference drug) (Figure 6).
Lysine showed high inhibition against DPPH activity with the low IC_{50} value of 7.60±0.31 µg/mL while cyclodextrin, Verb-Lysine, Verb-Cyclodextrin and acarbose (reference drug) revealed DPPH IC_{50} values that compared *(p>0.05)* well with that of verbenone (Table 3).
Lysine, cyclodextrin, Verb-Lysine, Verb-cyclodextrin and acarbose showed high inhibition against H_2O_2 concentration with low IC_{50} values of 6.82±0.34, 6.52±0.16, 7.32±0.26, 7.49±0.16 and 6.70±0.20 µg/mL respectively (Table 3).
Cyclodextrin showed the least inhibition against OH activity with the highest IC_{50} value of 275.97±44.84 µg/mL. However, acarbose showed the highest inhibition against OH activity with the lowest IC_{50} value of 11.36±0.88 µg/mL. The OH IC_{50} values for lysine, verb-lysine verb-cyclodextrin are 144.68±9.76, 107.49±18.07 and 59.06±14.95 µg/mL respectively (Table 3).

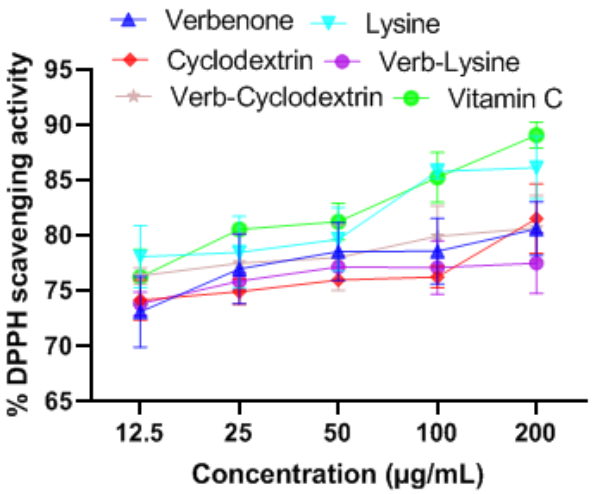

Figure 4: *In vitro* DPPH scavenging activity by verbenone enhanced with selected solubilizing compounds
Values are mean ± SEM

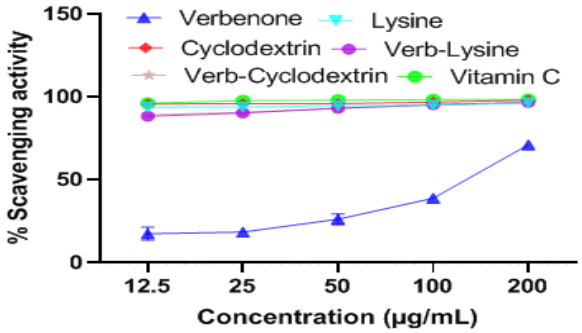

Figure 5: *In vitro* H_2O_2 scavenging activity by verbenone enhanced with selected solubilizing compounds
Values are mean ± SEM

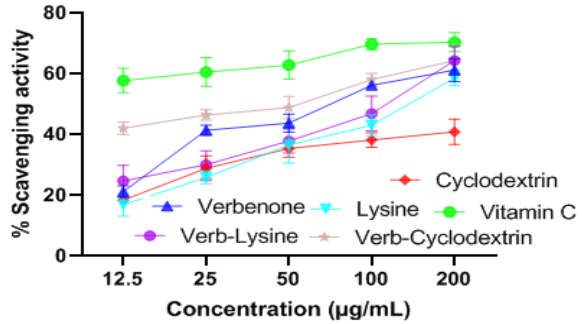

Figure 6: *In vitro* ·OH scavenging activity by verbenone enhanced with selected solubilizing compounds
Values are mean ± SEM

Table 3: IC_{50} values (µg/mL) for *in vitro* antioxidant effects of verbenone enhanced with selected solubilizing compounds

Groups	IC_{50} values (µg/mL)		
	DPPH	H_2O_2	·OH
Verbenone	8.97±0.55^{a}	142.63±11.11^{a}	73.73±9.62^{a}
Lysine	7.60±0.31^{b}	6.82±0.34^{b}	144.68±9.76^{b}
Cyclodextrin	8.52±0.36^{ab}	6.52±0.16^{b}	275.97±44.84^{c}
Verb-Lysine	8.63±0.38^{ab}	7.32±0.26^{b}	107.49±18.07^{b}
Verb-Cyclodextrin	8.35±0.20^{ab}	7.49±0.16^{b}	59.06±14.95^{a}
Acarbose	8.35±0.26^{ab}	6.70±0.20^{b}	11.36±0.88^{d}

Values are mean ± SEM. Significant difference are indicated using different superscript at $p<0.05$.

One of the antihyperglcaemic and antioxidative therapeutic strategies for glucose and oxidant regulation is the reduction in gastrointestinal glucose production by inhibiting carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase in the gastrointestinal tract, with related reduction of intestinal glucose absorption, and generation of enzymic and non-enzymic antioxidants thereby lowering the postprandial blood glucose levels and oxidant /free radical concentration (Nguyen *et al*., 2023). The reduction in glucose concentration by verbenone, lysine, cyclodextrin and metformin at 30 - 120 minutes suggest antihyperglycaemic effect of the compounds which may have acted by stimulating glucose utilization by peripheral tissues or increasing insulin production by the pancreas from regenerated β-cells (Tijjani & Imam, 2021). The reduction in percentage blood glucose level observed in all examined compounds (verbenone, lysine, cyclodextrin, verb-lysine and verb-cyclodextrin) from 0 - 120 minutes may be adduced to direct stimulation of the insulin release via the enteroinsular axis or reduced glucose uptake or combined effect of the two mechanisms. This is in accordance with the report by Bashir and Tijjani (2022).

α-amylase and α-glucosidase inhibitors delay the action of α-amylase and α-glucosidase enzymes to digest carbohydrate and prolong the overall carbohydrate digestion time thereby lowering the absorption of glucose and consequently reducing the postprandial plasma glucose. This has been employed as an oral antihyperglycemic drug especially in persons with hyperglycaemia (Oladipo *et al.,* 2024). Therefore, the increase in *in vitro* α-amylase inhibitory activity by verbenone at 12.5 µg/mL concentration when compared with other solubilizing compounds suggest that verbenone would inhibit the activity of α-amylase from hydrolyzing the polysaccharide (starch) to oligosaccharide (dextrin), thereby prolonging the overall carbohydrate digestion time. Consequently, this inhibition will lower glucose release. The increase in *in vitro* α-glucosidase inhibitory activity by all compounds at all concentrations in a manner comparable with acarbose suggest that all the verbenone enhanced solubilizing compounds would actively inhibit α glucosidase activity in hydrolyzing maltose to glucose, thereby delaying the overall carbohydrate digestion time, thus causing a reduction in the rate of glucose absorption and consequently reducing postprandial plasma glucose rise (Singharoy *et al*., 2024). Therefore, the ability of the verbenone and its linked solubilizing compounds to inhibit *in vitro* activities of α amylase and α glucosidase indicate that they can be explored as oral antihyperglycemic agent owing to their ability to inhibit intestinal glucose digestion and absorption. The increase observed in the present study is in agreement with the report of Jaber (2023) who reported similar increase in *in vitro* α amylase and α-glucosidase inhibitory activities.

Diabetes has been associated with several metabolic complications caused by oxidative stress. Free radicals especially reactive oxygen species (ROS) has been implicated in a lot of degenerative diseases particularly diabetes mellitus (Martemucci *et al.,* 2022). Furthermore, lipid peroxide-mediated tissue damage has been observed in the development of hyperglycaemia. The increased lipid peroxidation leads to cellular infiltration, islet cell dysfunction and destruction in diabetes (Jideani *et al*., 2021).

1,1-diphenyl-2-picrylhydrazyl is key antioxidant chemical for measurement of antioxidant properties of substances. It is a stable free radical that is used in assay to determine the antioxidant capacity of compounds, foods, beverages, and herbal extracts (Rahman *et al*., 2015). DPPH assay method exhibited the highest scavenging activity in all compounds

examined. Verbenone and its linked solubilizing compounds have a higher percentage of inhibition of DPPH radical scavenging activity and high total antioxidant capacity. The high scavenging potential of verbenone and its linked solubilizing compounds could also occur when the compounds donates hydrogen to DPPH, reducing its colour in solution from violet to yellow (Gulcin and Alwasel, 2023).

Hydrogen peroxide is a molecule that leads to generation of free radical via chemical reactions like Fenton chemical reactions and Habe-Weiss reactions. The free radical leading that has been generated leads to oxidative stress (Keser *et al*., 2012). The high percentage *in vitro* H_2O_2 scavenging activity of verbenone at all concentrations may be adduced to its capacity to facilitate reduction in hydrogen peroxide to prevent cell damage (Keser *et al*., 2012). The high H_2O_2 scavenging activity could have also occurred through its decomposition into water and oxygen following breakage of oxygen-oxygen bond which releases free radicals that are highly reactive with other biomolecules.

Hydroxyl radical (OH) is a strong oxidizing free radical that leads to lipid peroxidation through hydrogen abstraction technique. It generates free radicals by Fenton chemical process through interaction between hydrogen peroxide, iron or copper ions, and a reducing agent (Lyngsie *et al*., 2018). The high percentage of hydroxyl radical scavenging activity by verbenone in the present study may have occurred by accumulation of higher levels of simple organic compounds by verbenone, which could have occured when plant-derived sugars form less harmful sugar radicals during Fenton reactions (Lipinski, 2011).

CONCLUSION

The study indicated that enhancing verbenone with solubilizing compounds improved its antihyperglycemic properties in glucose loaded mice, increased inhibitory activities of α amylase and α glucosidase as well as increased *in vitro* antioxidant scavenging activities.

Conflict of interest: The author declares no conflict of interest.

Acknowledgments: The authors appreciate the National Open University of Nigeria, Abuja, Nigeria and TETFund Nigeria Institution Based Research (IBR) grant(NOUN/DRA/TETFUNDAW/VOL1/003/2024/IBR) for funding the research work.

REFERENCES

Ado, A. M., Mahmoud, F. A., Tijjani, H., Ibrahim, U., Tijani, S., Alka, S., Olatunde, A., Kura, A. U., Mohammed, A., Saliu, O. A., Idowu, O. A. and Ebhodaghe, F. E. (2024). Verbenone and L-arginine attenuate oxidative stress in streptozotocin-nicotinamide induced diabetic mice. *Tropical J. of Nat. Product Res.*, 8(9): 8490-8497. https://doi.org/10.26538/tjnpr/v8i9.35/

Andrade-Cetto, A., Becerra-Jimennez, J. and Cardenes, R. (2008). Alpha glucosidase inhibiting activity of some Mexican plants used in treatment of type 2 diabetes mellitus. *J. of Ethnopharmacology*, 116, 27-32. DOI: 10.1016/j.jep.2007.10.031

Asmat, U., Abad, K. and Ismail, K. (2016). Diabetes mellitus and oxidative stress – A concise review. *Saudi Pharmaceut. J.*, 24(5), 547–553. https://doi.org/10.1016/j.jsps.2015.03.013/

Attama, S. C., David, S. C., Egunleti, P. F., Okwelum, N. and Obetta, T. U. (2023). Antioxidant and antidiabetic effects of aqueous extract of *Senna alata* on alloxan-induced diabetic rats. *Sokoto Journal of Veterinary Sciences*, 21(2), 65-73. DOI: 10.4314/sokjvs.v21i2.3

Bashir, K. U. and Tijjani, H. (2022). Glucose lowering effects and *in vitro* α-amylase and α-glucosidase inhibitory potential from aqueous extract of *Adansonia digitata* (Baobab) seed. *Medical Sciences Forum*, 14(1), 66. https://doi.org/10.3390/ECMC2022-13239

Bhatti, J. S., Sehrawat, A., Mishra, J., Sidhu, I. S., Navik, U., Khullar, N., Kumar, S., Bhatti, G. K. and Reddy, P. H. (2022). Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. *Free Radical Biology and Medicine*, 184, 114-134. https://doi.org/10.1016/j.freeradbiomed.2022.03.019/

Binjawhar, D. N., Alhazmi, A. T., Jawhar, W. N. B., Saeed, W. M. and Safi, S. Z. (2023). Hyperglycaemia-induced oxidative stress and epigenetic regulation of ET-1 gene in endothelial cells. *Frontiers in Genetics*, 14, https://doi.org/10.3389/fgene.2023.1167773/

Gudise, V., Chowdhury, B. and Manjappa, A. S. (2021). Antidiabetic and antihyperlipidemic effects of *Argyreiapierreana* and *Matelea denticulata*: Higher activity of the micellar nano-formulation over the crude extract. *J. Tradit. Compliment Med*., 11, 259-267. 10.1016/j.jtcme.2020.08.001

Gulcin, I. and Alwasel, S. H. (2023). DPPH Radical Scavenging Assay. *Processes, 11*, 2248. https://doi.org/10.3390/pr11082248

International Diabetes Federation (IDF). In: *IDF Diabetes Atlas, 10th edn. Brussels, Belgium*, International Diabetes Federation, 2021.

Jaber, S. A. (2023). *In vitro* α-amylase and α-glucosidase inhibitory activity and *in vivo* antidiabetic activity of *Quercus coccifera* (Oak tree) leaves extracts. *Saudi J. of Biol. Sci.*, 30(7), 103688. https://doi.org/10.1016/j.sjbs.2023.103688

Jideani, A. I. O., Silungwe, H., Takalan, T., Omolola, A. O., Udeh, H. O. and Anyasi, T. A. (2021). Antioxidant-rich natural fruit and vegetable products and human health. *Int. J. Food Prop*., 24(1), 41–67. https://doi.org/10.1080/10942912.2020.1866597

Keser, S., Celik, S., Turkoglu, S., Yilmaz, O. and Turkoglu, I. (2012). Hydrogen peroxide radical scavenging and total antioxidant activity of hawthorn. *Chem J*., 2(1), 9–12.

Kokil, G. R., Veedu, R. N., Ramm, G. A., Prins, J. B., Parekh, H. S. (2015). Type 2 diabetes mellitus: limitations of conventional therapies and intervention with nucleic acid-based therapeutics. *Chem. Rev*., 115(11), 4719–4743. https://doi.org/10.1021/cr5002832

Kuppusamy, A., Muthusamy, U., Swashanmugum, T., Subhadradevi, V., Kalyanasubramaniam, R. and Sambathkumar, R. (2011). *In vitro* (α-Glucosidase and α-Amylase) inhibition and in vivo antidiabetic property of Phytic acid (ip_6) in Streptozotocin-Nicotinamide-induced type 2 Diabetes mellitus (NIDDM) in rats. *J. of Complem. and Integrative Med.*, 8, 1553-3840. DOI: 10.2202/1553-3840.1483

Lipinski, B. (2011). Hydroxyl radical and its scavengers in health and disease. *Oxid. Med. Cell Longev*., 809696. DOI: 10.1155/2011/809696

Lyngsie, G., Krumina, L., Tunlid, A. and Persson, P. (2018). Generation of hydroxyl radicals from reactions between a dimethoxyhydroquinone and iron oxide nanoparticles. *Sci. Rep.*, 8, 10834. https://doi.org/10.1038/s41598-018-29075-5

Mander, S., Kim, D. H., Nguyen, H. T., Yong, H. J., Pahk, K., Kim, E. Y., Lee, K., Seong, J. Y., Kim, W-K., Hwang, J-I. (2019). SP-8356, a (IS)-(-)-verbenone derivative, exerts *in-vitro* and *in-vivo* anti-breast cancer effects by inhibiting nf-ʁb signaling. *Sci. Rep*., 9(6595), 1–12. DOI: 10.1038/s41598-019-41224-y

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. https://doi.org/10.3390/oxygen2020006

McCune, L. M. and Johns, T. (2002). Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest. *Journal of Ethnopharmacology*, 82, 197-205. 10.1016/s0378-8741(02)00180-0

Nguyen, M. T., Thi, B. H. B., Maskey, S., Tran, M. D. and Nguyen, Q. V. (2023). In vitro and in vivo antioxidant and antihyperglycemic potentials of phenolic fractions of *Syzygium zeylanicum* (L.) DC trunk-bark. *Food Sci. Nutr*., 11(7), 3875-3884. DOI: 10.1002/fsn3.3373.

Oladipo, S. D., Luckay, R. C. and Olofinsan, K. A. (2024). Evaluating the antidiabetes and antioxidant activities of halogenated Schiff bases derived from 4-(diethylamino) salicylaldehyde: *in vitro* antidiabetes, antioxidant and computational investigation. *Sci. Rep.*, 14, 27073. https://doi.org/10.1038/s41598-024-78460-w

Rahman, M. M., Islam, M. B., Biswas, M. and Khurshid-Alam, A. H. M. (2015). *In vitro* antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. *BMC Res. Notes*, 8(1), 621. https://doi.org/10.1186/s13104-015-1618-6

Rappaport, N. G., Owen, D. R. and Stein, J. D. (2001). Interruption of semiochemical-mediated attraction of *Dendroctonus valens* (Coleoptera: *Scolytidae*) and selected nontarget insects by verbenone. *Environ Entomol*., 30, 837–841. https://doi.org/10.1603/0046-225X-30.5.837

Ruch, R. J., Cheng, S. J. and Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, 10, 1003–1008. DOI: 10.1093/carcin/10.6.1003

Singharoy, S., Pal, D., Das, S. and Ghosh, D. (2024). *In vitro* anti-diabetic and anti-oxidative evaluation of hydro-methanol bark extract of *Bauhinia acuminata* (L.). *Tropical Journal of Natural Product Research*, 8(4), 6932-6939. https://doi.org/10.26538/tjnpr/v8i4.26/

Smirnoff, N. and Cumbes, Q. J. (1989). Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry*, 28, 1057–1060. https://doi.org/10.1016/0031-9422(89)80182-7

Song, B. A., Liu, X. H., Yang, S., Hu, D. Y., Jin, L. H. and Zhang, H. (2005). Synthesis and anticancer activity of 234-trimethoxyacetophenoxime ester containing benzothiazole moiety. Chin J Chem., 23: 1236–1240.DOI: 10.1002/cjoc.200591236

Tijjani, H. (2022). L-arginine improves the solubility of verbenone in polar and non-polar solvent *in vitro*. *Academia Letters*, 4811. https://doiorg/1020935/AL481125

Tijjani, H. and Imam, S. A. (2021). Inhibition of haemoglobin glycosylation, glucose uptake and *in vitro* antidiabetic activities of solvent fractions from *Daucus carota* seed. *Ann. Sci. Technol.*, 6(1), 26–33. DOI: 10.2478/ast-2021-0004

Tijjani, H., Danyaro, A. M., Olatunde, A. and Kura, A. U. (2022). Antihyperglycemic activity of verbenone and L-arginine in nicotinamide-streptozotocin-induced diabetic mice: *in vitro* and *in vivo* studies. *Beni-Suef. Univ. J. Basic Appl. Sci*., 11, 94. https://doi.org/10.1186/s43088-022-00271-7

Tijjani, H., Ibrahim, U., Tijani, S., Alka, S., Olatunde, A., Kura, A. U., Gagman, H. A., Saliu, O. A., Idowu, O. A. and Ebhodaghe, F. E. (2023). Verbenone and L-arginine from *Daucus carota* seeds attenuate oxidative stress in streptozotocin-nicotinamide (STZ-NAD)-induced diabetic mice: Evidence from *In vitro* and *In vivo* Studies.biorXiv, 1-19. https://doi.org/10.1101/2023.09.28.559552/

Yang, L. and Hu, L. (2023). SP-8356: A novel verbenone derivative exerts *in vitro* anti-non-small cell lung cancer effects, promotes apoptosis via the P53/MDM2 axis and inhibits tumor formation in mice. *Cell J*., 25(12), 839-846. DOI: 10.22074/cellj.2023.2008708.1385.