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BIOACTIVE COMPOUNDS IN METHANOL EXTRACT OF *Newbouldia laevis* LEAF INHIBIT ALPHA-GLUCOSIDASE *IN SILICO* AND ENHANCE ANTIOXIDANT STATUS IN FRUCTOSE/ALLOXAN-INDUCED TYPE 2 DIABETIC RATS.

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ABSTRACT

Newbouldia laevis (NL) has been folklorically known to be used for managing diabetes mellitus. Its mechanism of action against this disease has not been fully proven. **T**he study was therefore designed to investigate the effect of NL bioactive compounds on diabetic parameters *in vivo* and *in silico*. For *in vivo* study, twenty-five rats divided into five groups (n = 5) were given 20% fructose solution for two weeks and grouped as follows: normal control (NC) received 10ml/kg olive oil; diabetes was induced in groups 2 (Diabetic control- DC), 3, 4 and 5 using 80mg/kg alloxan, and they were administered 10ml/kg olive oil, 250mg/kg, 500mg/kg MENL, and 5mg/kg glibenclamide, respectively. Methanol extract of NL (MENL) was characterized using Gas Chromatography Mass Spectrometry (GC-MS). Molecular docking was done on bioactive compounds obtained from GC-MS analysis against *α*-glucosidase to unravel probable mechanism of action of the extract. Serum insulin and antioxidant were determined using ELISA and spectroscopy techniques respectively. As against DC, blood glucose level was significantly low while insulin was high in MENL treated groups. Antioxidant increased significantly in MENL treated groups compared to DC. Four hit molecules inhibited α-glucosidase more than acarbose. It could be inferred that bioactive compounds in NL may exhibit their normoglycemic effect by inhibiting α-glucosidase, improving insulin sensitivity and enhancing antioxidant status.

Keywords: Alpha glucosidase, *Newbouldia laevis*, antioxidant, type 2 diabetes, insulin

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INTRODUCTION

Diabetes mellitus (DM) is one of the most conventional non-communicable diseases universally [1]. It has been discovered to be one of the leading causes of global mortality [2]. The global burden of the disease shows that 425 million people are living with diabetes mellitus: while 1.5 million persons loss their lives to the disease each year especially from low-middle income society. Unfortunately, about 50% of the diabetic individuals are unaware because of ignorance, lack of access to medical facilities and poverty. Late diagnosis can lead to amputation, diabetic nephropathy, male reproductive dysfunction due to tissue wastage among others [3].

Signs of DM comprises of excessively high blood glucose, recurrent urination, thirst, hunger, emaciation, and at times blurry vision. DM is categorized into Type 1 and type 2 DM. The former is triggered by absolute insulin pancreatic islet cells damage, while the latter is usually due to incidence of insulin resistance and insufficient compensatory insulin release response [4]. Ketoacidosis, a condition associated with elevated synthesis of ketone bodies due to lack of cell's access to glucose, occurs because of ionization of these metabolites leading to release of excess hydrogen ion into the cell. Insulin resistance which is one of the major causes of type 2 DM has been associated with inflammatory cytokines in the plasma and high hydrolysis of fats resulting into dearth of glucose supply into the cell, increased fat degradation and unnecessary elevated glucose supply from the hepatocytes. Type 2 DM stems from the interplay of genetic (e.g. imbalance crosstalk between nervous and endocrine systems), environment (toxicants which triggers inflammatory cytokines) and lifestyle factors such as *ad libitum* consumption of alcohol, overnutrition [5, 6]. Untreated diabetic condition is known to easily prone the patients to certain secondary complications or even untimely death, which underscores the need for early diagnosis. Drugs like sulphonylurea, biguanides, alpha glucosidase inhibitors are generally used in the treatment of DM. However, the prolong use of these medications are not without side effects which include hypoglycemia, blurry vision, diarrhea, nausea, low libido among others. These setbacks necessitated the search for complement or substitute from naturally occurring source such as plants. *Newbouldia laevis* is a good plant which has been attested for its folkloric use management of diabetes mellitus in Nigeria [7].

***Newbouldia laevis* (NL)**

This plant, a member of Bignoniaceae family, is commonly found in African countries (Nigeria, Ghana, and Togo etc). For instance, locally in Nigeria, it is called *ogilisi* (Hausa), *aduruku* (Igbo) and *akoko* (Yoruba) [7]. Some tribes in the country refer to it as fertility tree [8].

The leaf of NL has been known to be used folklorically for treatment and management of various ailments such as headache, induction of labour, malaria, diabetes mellitus [9]. Reports have shown that NL contains various phytoconstituents like flavonoids, alkaloids, tannin, saponin, polyphenols amongst others, which have assisted in exhibiting phytotherapeutic effects observed with this plant traditionally. Although it has been established that NL leaf could lower blood glucose, the mechanism of performing this function has not been fully unraveled [10]. It was also deemed necessary to investigate the activity of potential bioactive compounds against their molecular target (α-glucosidase) using *in silico* studies to identify the compounds that inhibit the enzyme; this would help in choosing the most potent compounds for further *in vitro* and *in vivo* studies. Therefore, the research was aimed to assess the mechanism of normoglycemic action of NL leaf via *in vivo* and *in silico* studies.

METHODS

Collection and Preparation of Plant Material

Newbouldia laevis leaves were obtained from Iya Sabo area, Ondo and it was authenticated from Herbarium unit, University of Medical Sciences, Ondo State with specimen number UNIMED-PBTH/042.

It was washed, air-dried at room temperature (28-30 ^{0}C) for two weeks and pulverized using blender. The powdered leaves were kept at room temperature in a clean jar.

The powdered leaves were soaked in methanol (1:10 w/v) for 72 hours because report showed that methanol extract exhibited highest antioxidant activity among others *in vitro* [8]. after which it was sifted via sterile Whatman number one filter paper. The filtrate was concentrated using rotary evaporator at reduced pressure and low temperature.

Ethical Approval

The Ethical Research Committee for Animal Care, University of Medical Sciences, Ondo, Ondo State, granted approval for the study. UNIMED-AREC/Apv/2023/015 was the authorized number.

Experimental Animals

Wistar rats (25) of weight 100-120g were purchased from Ogbomoso, Oyo State, Nigeria. The experimental sample size was determined via power analysis [11]. They were kept in ventilated cages

with iron nettings in 12 hours light/darkness cycle. Wood shaven used as the beddings were changed daily to prevent contamination and infections from the environment. They were made to adapt to the environment for a period of one week and fed with rat chows and water without restriction. The experimental animals were assigned into five groups (n=5).

Experimental Design

Animals was assigned into groups as follows**:**

Group 1: Non-diabetic control rats + 10ml/kg olive oil (as vehicle)
Group 2: Diabetic control rats + olive oil
Group 3: Diabetic + 250mg/kg methanol *Newbouldia laevis* leaf extract
Group 4: Diabetic + 500mg/kg methanol *Newbouldia laevis* leaf extract
Group 5: Diabetic + 5mg/kg glibenclamide.
The doses were determined by considering previously reported LD50 for methanol extract of *Newbouldia laevis* leaf which was >5000 mg/kg [12].

Induction of Diabetes and Treatment

Type 2 DM was induced in rats using 20% fructose (1 week) in water and thereafter, a dose of 80mg/kg Alloxan was administered intraperitoneally with slight modification of Udumula's method [13]. After three days the animals were investigated for their blood glucose levels using a Fine Test glucometer. Rats with 250 mg/dl of blood glucose were considered diabetic and used for the experiment. The diabetic rats were treated for thirty days using aqueous fraction of *Newbouldia laevis* by oral gavaging.

Isolation of Animal Tissue

After an overnight fast, the animals were sacrificed by jugular puncture. The liver and blood sample were collected. Thereafter blood was centrifuged at 3,000 rpm for about 5 minutes using the refrigerated centrifuge RC650s and the serum obtained were preserved in a refrigerator until use.

Preparation of Homogenate

The isolated liver was weighed and homogenized using a homogenizer with Teflon (BOCSH, PSB 570-2) and homogenizing flask. The homogenates were distilled 1:5 (w/v of sample and buffer, respectively) with phosphate buffer (pH 7.4). Post-mitochondrial fraction of homogenates was obtained by centrifugation at 10,000 rpm. The supernatants were collected and stored at a temperature of -8 ^{o}C until required for use.

Homeostasis model assessment of insulin resistance (HOMA-IR) and pancreatic function (HOMA- β)

These were used to assess insulin resistance and sensitivity as observed in type 2 DM. The parameters were determined using formulae as stated by Matthew *et al*., [14].

Biochemical investigation

Insulin concentration

Serum insulin status was evaluated using ELISA kit in accordance to manufacturer's instruction.

Antioxidant enzyme assays
Catalase activity

Hydrogen peroxide (1.48 ml of 19 mM solution) and 25 µl of sample were dispensed into quartz cuvette, mixed by rapid inversion and estimated spectrophotometrically. For a period of 300s at 1 min interval, change in absorbance was determined at λ_{240nm} [15].

Calculation

Catalase activity

$$= \frac{\Delta A240/min \times \text{reaction volume} \times \text{dilution factor}}{0.0436 \times \text{sample volume} \times \text{mg protein/ml}}$$

$= \mu mole\ H_2O_2/min/mg\ protein$

Glutathione Peroxidase activity

Into the test tube were dispensed in sequential order 1 ml (0.1M) phosphate buffer, 0.2 ml NaN_3, 0.4 ml (4mM) GSH, 0.2 ml (2.5mM) H_2O_2 and 1 ml sample (added last). The reaction mixture was incubated for 180 seconds at physiological temperature following addition of 1 ml (10%) trichloro acetic acid and the final mixture was spun at 3000 rpm for 5 min. To 0.5 ml of the supernatant, 1 ml (0.3M) K_2HPO_4 and 0.5 ml DTNB were dispensed into the medium and the absorbance was

taken after blanking with 0.5 ml distilled water, 1 ml K_2HPO_4 and 0.5 ml DTNB a λ_{421nm} [16].

Reduced Glutathione level

Sample (0.6 ml) was mixture with 0.6 ml of precipitating (4% sulphosalicylic acid) solution by vortexing and thereafter spun at 4000 rpm for 5 min. Afterward, 0.75 ml supernatant was reacted with 2.25 ml of Ellman's reagent. Absorbance of the total reaction volume was read at λ_{412nm} after blanking [17].

Glutathione S transferase

To 30µl sample was added 20mM CDNB (150µl), 30µl GSH (0.1M), and 2.79ml phosphate buffer (0.1M, pH 6.5). They were gently mixed and left to run for 3 min at 60 seconds interval using spectrophotometer at wavelength 340nm [18].

Thiobarbituric acid reactive substance

Sample (0.2ml), 0.8ml Tris-KCl (0.15M) and 0.25 ml TCA (30%) were added. Afterward, 0.25 ml TBA (0.75%) was added and heated in water bath at 90°C for one hour. It was then left to cool and spun at 3,000 rpm for 600s. Supernatant absorbance was estimated after blanking with dH_2O at λ_{532nm} [19].

Calculation

The MDA status was evaluated via an extinction coefficient of 0.156 µM-1cm-1 [20].

$$\text{Lipid peroxidation (nmole MDA/mg protein)} = \frac{\text{Absorbance} \times \text{volume of mixture}}{E_{532nm} \times \text{volume of sample} \times \text{mg protein/ml}}$$

SOURCES OF LIGANDS

The structure bioactive compounds of NL and other standard drug inhibitors of proteins of interest were retrieved (in structure-data file, SDF, format) from the ligand database; PubChem (https://pubchem.ncbi.nlm.nih. gov/).

PROTEIN PREPARATION

Crystal structures of the proteins were retrieved from the RCSB-PDB database (https://www.rcsb.org/). Protein of interest retrieved was α-glucosidase (PDB ID: 3WY1, 123.08kDa). Using Biovia Studio Visualizer tools (2024), the target proteins were cleaned up before docking by removing all water molecules, inhibitors, co-crystallized compounds, and missing hydrogen atoms. Thereafter, the obtained pure protein targets were prepared using UCSF Chimera tools.

Ligand Preparation and Molecular Docking

Molecular docking analysis of all selected ligands against protein receptors was performed using the Pyrex software tool. The PDB and SDF formats of the proteins and ligands respectively were imported into their respective columns and the software was run. The binding affinities of the compounds for the target protein were recorded and ranked by their affinity scores. To compare *in silico* performance, the molecular interactions between the protein and the ligands with the highest binding affinity besides the standard inhibitor was viewed with Discovery Studio Visualizer, BIOVIA, 2024. Also, the binding of the ligands for the receptor and the drug with highest binding affinity using receptor cavity method in Discovery Studio Visualizer, BIOVIA, 2024.

STATISTICAL ANALYSIS OF DATA

The data collected were presented as mean ± standard deviation of three separate assays. One-way analysis of variance and Duncan's multiple Range Test were carried out. $p<0.05$ was regarded statistically significant.

Results and Discussion

Effect of Methanol extract of *Newbouldia laevis* leaf on Blood glucose and Serum insulin in type 2 diabetic rats.

Table 1 shows the status of blood glucose and insulin in type 2 diabetic rats treated with NL leaf for a period of four weeks. There was significant decrease in the blood glucose level of diabetic control group relative to normal control. However, there was no significant difference in groups treated with methanol extract of NL leaf in comparison with normal control. Similarly, there was significant decrease in insulin level in groups treated with methanol extract of NL and glibenclamide when compared to diabetic control group. Table 1 results prove that the plant extract possesses bioactive

compounds that could normalize elevated blood glucose and as well improve insulin secretion. Reports on some plant extracts have demonstrated normoglycemic effect on diabetic rats [21]. Studies have also shown that certain bioactive compounds in the chloroform fraction of *Ocimum gratissimum* leaf extract lowered blood glucose and normalized insulin status significantly in diabetic rats [22].

Effect of Methanol extract of *Newbouldia laevis* leaf on HOMA-IR and HOMA-β in type 2 diabetics rats.

Table 2 shows the result on effect of HOM-IR and HOMA-Beta values in type 2 diabetic rats treated with methanol extract of NL for a period of four weeks. There was a significant elevation in the HOMA-IR value of diabetic control relative to normal control. However, there was dose-dependent decrease in HOMA-IR values in the group treated with methanol extract of NL leaf and glibenclamide when compared with diabetic control. Furthermore, there was a significant decrease in the HOMA-β values of diabetic control compared to normal control. In contrast, there was a significant increase in the HOMA-β values of the groups treated with methanol extract of NL leaf and glibenclamide relative to diabetic control.

HOMA-IR is an index to measure insulin resistance while HOMA-β is an indicator to measure beta cell function. Table 2 result shows that *Newbouldia laevis* was able to prevent insulin resistance, as well as enhance beta cell function. It has been observed from previous research that oxidative stress and inflammation are precipitated by overwhelming reactive oxygen species in the cells. Inflammation that results from hyperglycemia is known to be involved in the pathogenesis of DM and its secondary conditions [23]. Beta cell death may also ensue from elevated ROS and inflammatory marker, thus impairing insulin synthesis and signaling. Defect in this pathway, alters glucose uptake into the muscle and other peripheral tissues [24]. Prevention of excessive ROS and inflammation can therefore impede insulin resistance and elevated blood glucose peculiar to T2DM.

Effect of methanol extract of *Newbouldia laevis* leaf on antioxidant status in type 2 diabetic rats.

Figures 1, 2, 3, 4 and 5 shows the result of catalase, GST, GPx activities, GSH and MDA levels in type 2 diabetic rats treated with methanol extract of NL leaf for period of four weeks respectively.

Figure 1 shows significant decrease in catalase activity in the diabetic control group relative to normal control. However, there was a significant increase in catalase activity in group treated with glibenclamide relative to normal control. The result also shows dose-dependent increase in group treated with methanol extract of NL in comparison to diabetic control.

Figure 2 shows a significant decrease in GST activity in diabetic control rats compared to normal control. However, there was a significant increase in GST activity in group treated with glibenclamide compared to normal control group, as well as dose-dependent increase in groups administered with methanol extract of NL relative to diabetic control.

Figure 3 shows a significant reduction in GPx level in diabetic control group compared to normal control. However, there was a significant dose–dependent increase in GPx activity in group treated with methanol extract of NL compared to normal control.

Figure 4 shows a significant increase in MDA level in diabetic control group relative to normal control. However, there was a significant decline in MDA level in group treated with methanol extract of NL and glibenclamide compared to normal control.

Figures 1, 2, 3, 4 and 5 revealed that *Newbouldia laevis* leaf extract possesses antioxidant potential that could scavenge free radical. Recent reports revealed the DPPH radical-scavenging potential of *Newbouldia laevis* [25]. Compelling evidence has shown that oxidative stress is one of the pathogenesis of diabetes mellitus because it can lead to impairment of β-cell of the pancreas which invariably would impede production of insulin. This could occur through formation of advanced glycation endproduct viz a viz NADPH oxidase pathway [26]. Enhanced antioxidant activities observed in this study suggests that the phytochemicals in the plant extract could scavenge free radicals generated in the course of the disease.

Thus, the presence of antioxidants protects the β-cells from oxidative damage peculiar to diabetes mellitus. It has been established that many phytoconstituents in plants possess ability to prevent accumulation of free radicals through directly scavenging or boosting the antioxidant status of the cell to cope effectively with the generated pro-oxidants. Recent evidence revealed that epigallocatechin gallate, an antioxidant from green tea expressed its anti-diabetic property by stabilizing pro-oxidant/antioxidant imbalance and mitochondrial dysfunction [27]. Moreover, reduced MDA status in the treated groups is consistent with ability of the extract to improve antioxidant status of the cells and it is indicative of low lipid peroxidation in the cells.

GC-MS analysis showed that the methanol of *Newbouldia laevis* contained 24 bioactive compounds

Molecular interaction of hit compounds with enzyme/receptor

The phytoconstituents of *Newbouldia laevis* were chosen for interaction analysis centred on their docking scores and complexes they form with α-glucosidase catalytic domain, relative to the standard compound, acarbose. The analysis revealed that these interactions included H-bonds, hydrophobic connections, and various types of bonding. Evaluation of the interactions demonstrated that both the phytoconstituents and acarbose exhibited strong binding affinities for the enzyme. Specifically, acarbose formed hydrogen bonds with the residues ASP^{282}, ASP^{404}, and ASP^{616}, alongside alkyl/π-alkyl connectivity with LEU^{650} and PHE^{649}. Additionally, acarbose exhibited an unfavourable donor-donor interaction with ALA^{289} within the active pocket of α-glucosidase (Figure 6).

The interaction of 1-(4-piperidinylcarbonyl) piperidine with the active site of α-glucosidase revealed several types of binding complexes, comprising conventional H-bonds with ARG^{606} and ASP^{404}, a C-H bond with ASP^{518}, and a π-alkyl bond with TRP^{418} and PHE^{649} (Figure 7). Gingerol exhibited hydrogen bonding with the enzyme through residues MET^{519} and ARG^{600}, carbon-hydrogen interactions with ASP^{616}, and alkyl/π-alkyl interactions LEU^{283} and TRP^{376}. Additionally, gingerol formed unfavourable donor-donor and acceptor-acceptor interactions with ARG^{281} and ASP^{518} (Figure 8). Carinol bound to the enzyme by forming hydrogen bonds with ASP^{282}, ASP^{404}, ASP^{616}, and HIS^{674}, along with π-anion complexing with ASP^{518} and alkyl/π-alkyl interactions with LEU^{283} and TRP^{613} (Figure 9). Finally, gamma-sitosterol demonstrated four alkyl/π-alkyl interactions with residues TRP^{376}, ALA^{555}, PHE^{649}, and LEU^{650}, as well as a carbon-hydrogen bond with ASP^{282} (Figure 10).

Alpha glucosidase is responsible for the breakdown of α-1,4-glucosidic linkage in carbohydrates in the intestine, thus paving way for its transportation across the intestinal epithelial cell into the blood leading to elevated blood glucose. From this study, piperidin 1-(4- piperidinylcarbonyl), a heterocyclic amine, was observed to strongly inhibit alpha glucosidase better than the reference drug. Previous report showed that piperidin derivatives, nojirimycin and deoxy nojirimycin, exhibited strong inhibitory effects on alpha glucosidase in *in vitro* study [28].

It is interesting that gingerol, a beta hydroxyl ketone, and an active compound in ginger is also present in *Newbouldia laevis* leaf. It was discovered from this study (Figure 8) that gingerol was able to inhibit the activity of alpha glucosidase, thus showing, its antidiabetic potential by delaying postprandial glucose absorption and preventing unnecessary drastic rise in blood glucose. Inhibition of alpha glucosidase was discovered to be its mechanism of action in preventing hyperglycemia. Research also showed that gingerol reduced blood glucose and attendant secondary complication of diabetes [29]. Carinol action against hyperglycemia can be attributed to inhibition of alpha glucosidase, although no previous research has shown this fact. Furthermore, gamma sitosterol inhibits alpha glucosidase as its mode of antidiabetic activity. Increasing evidence revealed that gamma sitosterol displayed antidiabetic activity in streptozotocin-induced diabetic rats by maintaining glucose homeostasis via improved skeletal muscle GLUT4, PPARγ expressions, insulin secretion and reduced insulin resistance [30, 31]. Improved skeletal muscle GLUT4 expression would enhance rapid uptake of glucose into the muscle for oxidation while PPARγ helps to facilitate consumption of

carbohydrates and fatty acid which would prevent insulin insensitivity.

CONCLUSION

This study shows that methanol extract of NL leaf was able to normalize blood glucose, increase insulin sensitivity and secretion as well as reduce insulin resistance. NL leaf extract exhibited significant free radical scavenging potential as shown in its ability to improve the antioxidant status of the treated rats. *In silico* study revealed that NL leaf may exhibit its anti-hyperglycemic effect by inhibiting α-glucosidase.

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Conflict of interest

There was no conflict of interest whatsoever.

Authors' contribution

AJ conceptualize the study, GO read the manuscript, OG wrote the manusript, ZS, SJ, ME, TM and MO fed the animals, carried out various assays and analysed the results.

REFERENCES

[1] Kaviarasan, S., Muniandy, S., Qvist, R., Chinna, K. and Ismail I.S. (2010). Is F2-isoprostane a biological marker for the early onset of Type 2 diabetes mellitus? *International Journal of Diabetes for Developing Countries*, 30, 165-170.

[1] Amos, A. F., McCarty, D.J., and Zimmet P. (1997). The rising global burden of diabetes and its complications: Estimates and projections to the year 2010. *Medicine*, 14, S7-S85.

[3] Nwafor, C.E., Edeogu, J., Stanley, R., Enyichukwu, B., Ogomegbunam, M. (2024). Prevalence of Diabetes Mellitus Among Adult Population Within a Southern Nigerian Community. *Eur. J. Med. Health Res*., 2(1):131-7.

[4] Dendup, T., Feng, X., Clingan, S., Astell-Burt, T. (2018). Environmental risk factors for developing type 2 diabetes mellitus: a systematic review. Int J Environ Res Public Health 15(1).

[5] Chen, L., Magliano, D.J., Zimmet P.Z. (2011). The worldwide epidemiology of type 2 diabetes mellitus--present and future perspectives, *Nat. Rev. Endocrinol*., 8; 8(4):228-36.

[6] Amechina, O.O. (2003). Three minutes herbal treatment to reduce dental caries with *Newbouldia laevis* based extract, *American Journal of Undergraduate Research*, 2 (2):1-4.

[7] Iwu, M.M. (2000). Handbook of African medicinal plants. London CRC Press Inc. 19th Edition.

[8] Salemcity, A. J., Nwaneri-Chidozie, V. O., Adameh, E., Effiong M.E. (2020). Antioxidant and Free Radical Scavenging Activity of *Newbouldia laevis* Leaf extracts (*in vitro*). *Free Radicals and Antioxidants*, 10(1):10-15.

[9] Udumula, M.P., Mangali S., Kalra, J., Dasri, D., Goyal, S., Krishna, V., Bollareddy, S.D., Dhar, A. and Bhat A. (2021). High fructose and streptozotocin diabetic impairments are mitigated by indirubin-3-hydrazone via downregulation of PKR pathway in Wistar rats. *Sci. Rep*., 11:12924.

[10] Osigwe, C., Akah, P., Nworu, C., Okoye, T. and Tchimene, M. (2015) Antihyperglycemic Studies on the Leaf Extract and Active Fractions of *Newbouldia laevis* (Bignoniaceae). *Pharmacology & Pharmacy*, **6**, 518-532.

[11] Dell, R. B., Holleran, S,, Ramakrishnan R. (2002). Sample size determination ILAR J. 43:207–13.

[12] Itez, P., Aloh, G.S., Osuocha, K.U., Iwueke, A.V. and Chukwu, E.C. (2020). Ameliorative

potentials of methanol leaf extract of *Newbouldia laevis* on monosodium glutamate induced toxicity in female albino rats. *African Journal of Biological Sciences*. 2(3), 37-50.

[13] Udumula, M.P., Mangali, S., Kalra, J., Dasri, D., Goyal, S., Krishna, V., Bollareddy, S. D., Dhar, A. and Bhat, A. (2021). High fructose and streptozotocin diabetic impairments are mitigated by indirubin-3-hydrazone via downregulation of PKR pathway in Wistar rats. Sci. Rep. 11:12924.

[14] Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., Turner, R.C (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, 28:412–419.

[15] Claiborne, A. (1985). Catalase activity In: Greenwald RA (ED) Handbook of methods for oxygen radical research, CRC Press, Boca Raton, 283-284.

[16] Rotruck**,** J.T**.**, Pope**,** A.L**.**, Ganther**,** H.E**.**, Swanson**,** A.B**.**, Hafeman**,** D.G**.**, Hoekstra**,** W.G. **(**1973). Selenium: biochemical role as a component of glutathione peroxidase. *Science*, 9; 179 (4073):588-90.

[17] Beutler, E., Duron, O. and Kelly, B.M. (1963). Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.*, 61: 882-8.

[18] Habig, W., Pabst, M. and Jakoby, W. (1974). Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry, 249*, 7130-7139.

[19] Varshney, R. and Kale, R.K. (1990). Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. *Int. J. Radiat. Biol.*, 58 (5): 733-43.

[20] Adam, V.V. and Seregi, M. (1982). Receptor Dependent Stimulatory Effect of Noradrenaline on Na+/K+ ATPase in Rat Brain Homogenate. Role of Lipid Peroxidation. *Biochemical Pharmacology*, 31, 2231-2236.

[21] Fatima, M.T., ABhat, A., Nisar, S., Fakhro, K.A., Al-Shabeeb, A.A.S. (2023). The role of dietary antioxidants in type 2 diabetes and neurodegenerative disorders: An assessment of the benefit profile. *Heliyon*, 9; e12698.

[22] Salemcity, A.J, Olanlokun, J.O, Olowofolahan, A.O, Olojo, F. O, Adegoke, A.M, Olorunsogo O.O (2023). Reversal of Mitochondrial Permeability Transition Pore and Pancreas Degeneration by Chloroform Fraction of *Ocimum gratissimum* Leaf Extract in Type 2 Diabetic Rat Model. *Frontier in Pharmacology*, doi: 10.3389/fphar.2023.1231826. 2023; 1-14.

[23] Lovejoy, J.C. (2002). The influence of dietary fat on insulin resistance. *Curr Diab. Rep.*, 2 (5): 435-440.

[24] Dada A.A and Ajilore V.O., (2009). Use of ethanol extracts of *Garcinia kola* as fertility enhancer in female catfish Clarias garipinus broodskock, *Int. J. Fish and Aquacul.*, 1 (1): 5-10.

[25] Zafar, F., Asif, H.M., Shaheen, G. *et al*., (2022). A comprehensive review on medicinal plants possessing antioxidant potential. *Clin Exp Pharmacol Physiol*., 50(3): 205-217.

[26] Yi, X., Dong, M., Guo, N., Tian, J., Lei, P., Wang, S., Yang, Y. and Shi Y., (2023). Flavonoids improve type 2 diabetes mellitus and its complications: a review. *Frontiers in Nutrition*, 10:1192131.

[27] Liu, H., Guan, H., Tan, X., Jiang, Y., Li, F., Sun-Waterhouse, D, et al. (2022). Enhanced alleviation of insulin resistance via the IRS-1/Akt/FOXO1 pathway by combining quercetin and EGCG and involving miR-27a-3p and miR-96-5p. *Free Radic Biol Med*. 181:105–17.

[28] Rafiq, S., Kaul, R., Sofi, S. A., Bashir, N., Nazir, F., Ahmad, N, G. (2018). Citrus peel as a source of functional ingredient: A

review, *Journal of the Saudi Society of Agricultural Sciences,* 17(4), 351-358.

[29] Alharbi1, M. S., Alrashidi, T., Al-Harbi, M. S., Al Zahrani, E., Alkahlot, M. H., Aljaberi M. (2023). Assessment of Elderly Type 2 Diabetic Patients who Self-adjust Their Insulin Regimen in Al-Qassim Region. *Macedonian Journal of Medical Sciences,* 11(B): 519-523.

[30] Balamurugen R., Duraipandiyan, V., Ignacimuthu S., (2011). Antidiabetic activity of γ-sitosterol isolated from *Lippia nodiflora* L. in streptozotocin induced diabetic rats. *European Journal of Pharmacology*. 667(1–3): 410-418.

[31] Ramalingam, S., Packirisamy, M., Karuppiah, M., Vasu, G., Gopalakrishnan, R., Gothandam, K., Thiruppathi, M. (2020). Effect of β-sitosterol on glucose homeostasis by sensitization of insulin resistance via enhanced protein expression of PPRγ and glucose transporter 4 in high fat diet and streptozotocin-induced diabetic rats. Cytotechnology. 72(3):357-366.

Table 1: Effect of methanol extract of *Newbouldia laevis* leaf on Blood glucose and insulin in type 2 diabetic rats

	Blood Glucose Levels (mg/dl)	**Insulin (m/U/L)**
Control group	99 ± 1.7###	7.50 ± 2.7
Diabetic control + olive oil	384 ± 7.6***	6.12 ± 0.6*
Diabetes+ 250mg/kg methanol extract of *Newbouldia laevis*	222 ± 11.6**##	7.20 ± 2.5
Diabetes + 500mg/kg methanol extract of *Newbouldia laevis*	190 ± 13.1**##	8.89 ± 1.2
Diabetes + 5mg/kg Glibenclamide	122.5 ± 0.71*###	6.90 ± 0.9

#- Comparison of diabetic control to other groups

*- Comparison of normal control to other groups

Table 2: Effect of methanol extract of *Newbouldia laevis* leaf on HOMA- IR and HOMA-β in type 2 diabetic rats

	HOMA- IR	**HOMA-β**
Normal control	1.833 ± 0.0	75 ± 2.0####
Diabetic control + olive oil	5.80 ±. 01***	6.86 ± 0.2****
Diabetes + 250mg/kg methanol *Newbouldia laevis*	4.17 ± 0.3**#	16.30 ± 0.7**##
Diabetes + 500mg/kg methanol *Newbouldia laevis*	3.94 ± 0.6**#3	25.15 ± 0.8***##
Diabetes + 5mg/kg Glibenclamide	2.09 ± 0.0**##	41.75 ± 0.1***###

*** Test groups compared to normal control**

 # Test groups compared to diabetic control

Table 3: GC-MS analysis of methanol extract of *Newbouldia laevis*

Peak#	Retention Time	Area(A)	Area%	Height(H)	Height%	A/H	Name
1	6.139	1874481	3.79	1415326	5.6	1.3	2-Propanol, 1,1,1-trichloro-2-methyl-
2	10.154	648950	1.31	397496	1.57	1.6	9-Oxa-bicyclo [3.3.1] nonane-1,4-dio
3	10.643	676628	1.37	457302	1.81	1.5	Propylphosphonic acid, di(2-methylpentyl) ester
4	11.69	1936999	3.91	648111	2.57	3	2H-Pyran-2-one, tetrahydro-4-hydroxy-6-pentyl
5	12.164	2399103	4.85	1351493	5.35	1.8	1,7-Dioxaspiro [5.5] undec-2-ene
6	12.321	606910	1.23	366792	1.45	1.7	Cyclopentane, heneicosyl-
7	14.02	5091545	10.28	1154011	4.57	4.4	Succinic acid, cyclohexylmethyl ethyl ester
8	14.442	1010543	2.04	602413	2.39	1.7	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphen
9	14.615	724486	1.46	445559	1.76	1.6	E-6-Octadecen-1-ol acetate
10	15.334	906602	1.83	396331	1.57	2.3	Tetradecanoic acid, 12-methyl-, methyl ester
11	16.224	481760	0.97	367055	1.45	1.3	Hexadecanoic acid, methyl ester
12	16.583	2008410	4.06	526416	2.08	3.8	N, N'-Dibutylidene-hydrazine
13	16.797	820538	1.66	461162	1.83	1.8	Benzenepropanoic acid, 2,5-dimethoxy-
14	18.411	2323083	4.69	1467548	5.81	1.6	Hexadecanamide
15	18.461	1838560	3.71	710103	2.81	2.6	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrr
16	18.515	946317	1.91	558872	2.21	1.7	Piperidine, 1-(4-piperidinylcarbonyl)-
17	19.194	641512	1.3	452148	1.79	1.4	Gingerol
18	19.609	1761891	3.56	1180902	4.68	1.5	8,11-Octadecadienoic acid, methyl ester
19	19.662	16849302	34.03	10101175	39.99	1.7	9-Octadecenamide, (Z)-
20	19.781	630959	1.27	385760	1.53	1.6	Octadecanoic acid, 2,3-dihydroxypropyl ester
21	19.835	552413	1.12	390621	1.55	1.4	Octadecanamide
22	20.402	691814	1.4	360430	1.43	1.9	Carinol
23	20.668	799342	1.61	438209	1.73	1.8	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl
24	21.077	3284319	6.63	622395	2.46	5.3	gamma-Sitosterol
		49506467	100	25257630	100		

Table 4: Docking scores of ligand obtained from GCMS analysis

S/N	Ligand	Binding Affinity Alpha glucosidase	rmsd/ub	rmsd/lb
1	2-Propanol, 1,1,1-trichloro-2-methyl-	-4.2	0	0

2	9-Oxa-bicyclo [3.3.1] nonane-1,4-dio	-5.3	0	0
3	Propylphosphonic acid, di(2-methylpentyl) ester			
4	2H-Pyran-2-one, tetrahydro-4-hydroxy-6-pentyl	-5.9	0	0
5	1,7-Dioxaspiro [5.5] undec-2-ene	-5.7	0	0
6	Cyclopentane, heneicosyl-	-5.4	0	0
7	Succinic acid, cyclohexylmethyl ethyl ester	-5.6	0	0
8	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphen	-5.6	0	0
9	E-6-Octadecen-1-ol acetate	-5.2	0	0
10	Tetradecanoic acid, 12-methyl-, methyl ester	-4.9	0	0
11	Hexadecanoic acid, methyl ester	-4.8	0	0
12	N, N'-Dibutylidene-hydrazine	-4.8	0	0
13	Benzenepropanoic acid, 2,5-dimethoxy-	-5.3	0	0
14	Hexadecanamide	-5	0	0
15	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrr	-5	0	0
16	Piperidine, 1-(4-piperidinylcarbonyl)-	**-6.2**	0	0
17	Gingerol	**-6.4**	0	0
18	8,11-Octadecadienoic acid, methyl ester	-5.1	0	0
19	9-Octadecenamide, (Z)-	-5.5	0	0
20	Octadecanoic acid, 2,3-dihydroxypropyl ester	-5.2	0	0
21	Octadecanamide			
22	Carinol	**-7.2**	0	0
23	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl			0
24	gamma-Sitosterol	**-8.1**	0	0
25	Acarbose (Alpha glucosidase inhibitor)	**-6.1**	0	0

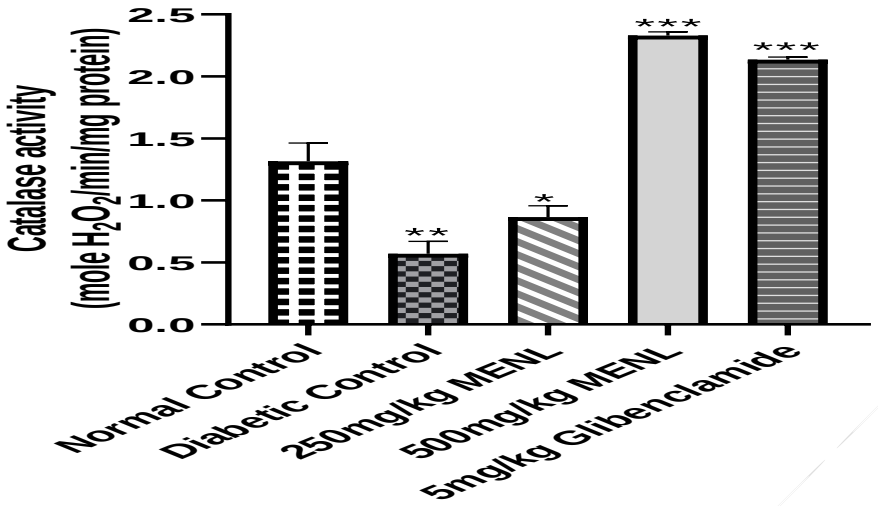


Figure 1: Effect of methanol extract of *Newbouldia laevis* leaf on catalase activity in type 2 diabetic rats

MENL: Methanol extract of *Newbouldia laevis*

***P*<0.05; ****P*<0.01; *****P*<0.001 (Test group vs Normal control)**

MENL: Methanol extract of *Newbouldia laevis*

***P*<0.05; ****P*<0.01; *****P*<0.001 (Test group vs Normal control)**

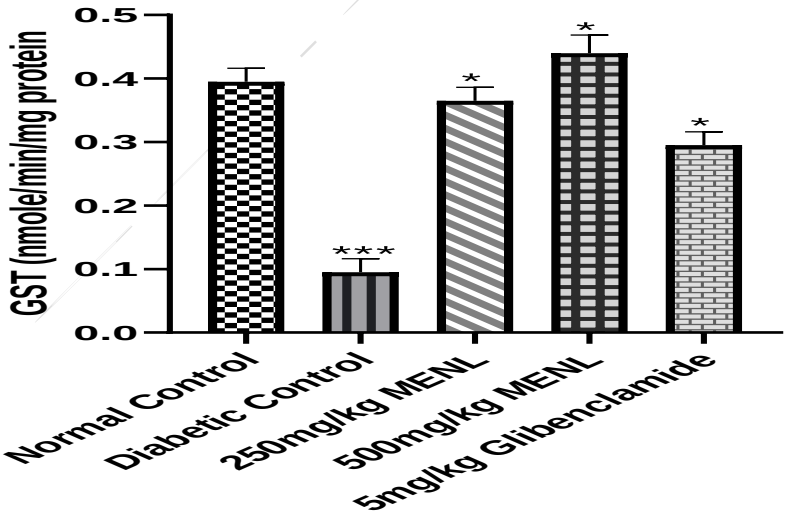


Figure 2: Effect of methanol extract of *Newbouldia laevis* leaf on GST activity in type 2 diabetic rats.

MENL: Methanol extract of *Newbouldia laevis*

***P*<0.05; ****P*<0.01; *****P*<0.001 (Test group vs Normal control)**

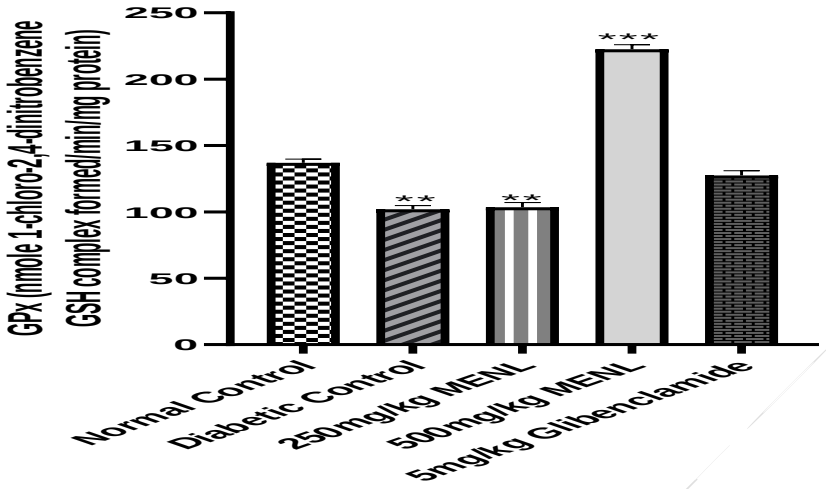


Figure 3: Effect of methanol extract of *Newbouldia laevis* leaf on GPx activity in type 2 diabetic rats.

MENL: Methanol extract of *Newbouldia laevis*

***P*<0.05; ****P*<0.01; *****P*<0.001 (Test group vs Normal control)**

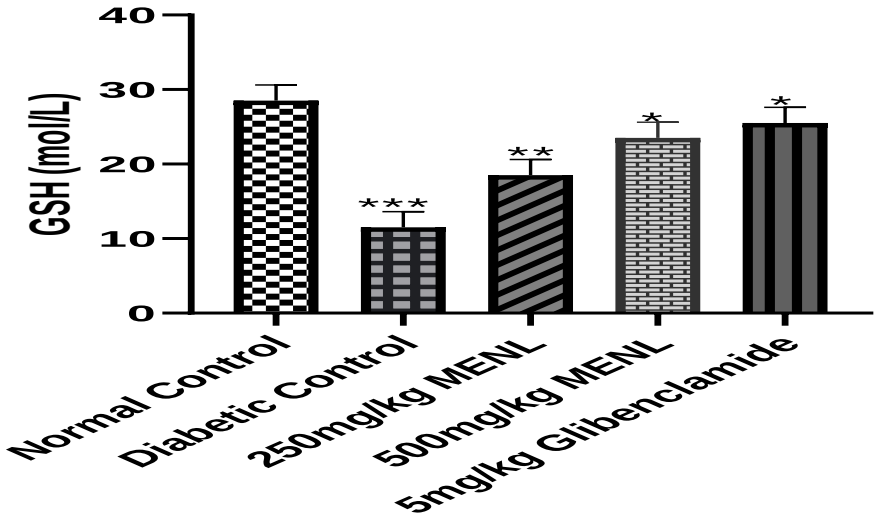


Figure 4: Effect of methanol extract of *Newbouldia laevis* leaf on GSH level in type 2 diabetic rats.

MENL: Methanol extract of *Newbouldia laevis*

***P*<0.05; ***P*<0.01; ****P*<0.001 (Test group vs Normal control)**

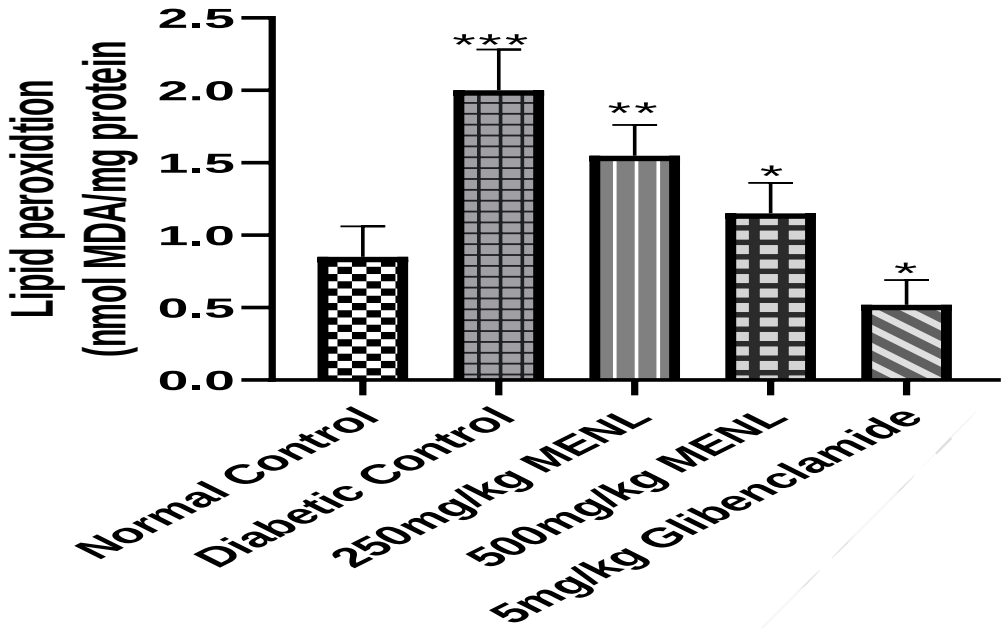
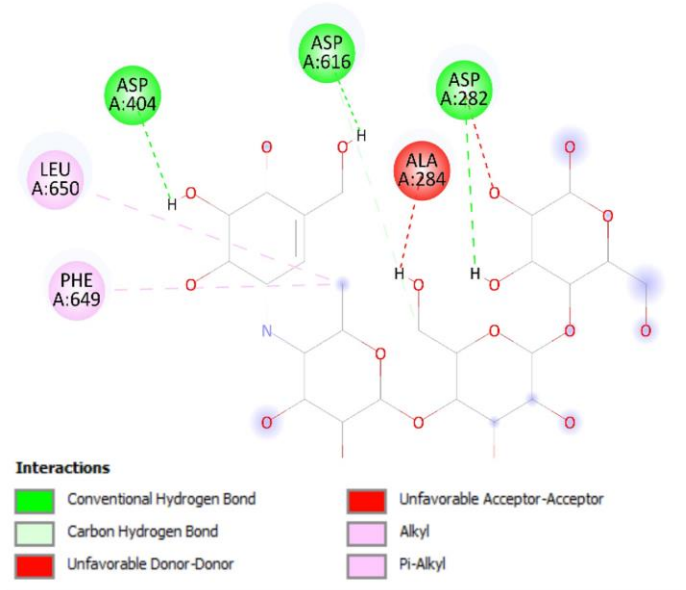


Figure 5: Effect of methanol extract of *Newbouldia laevis* leaf on Lipid Peroxidation type 2 diabetic rats.



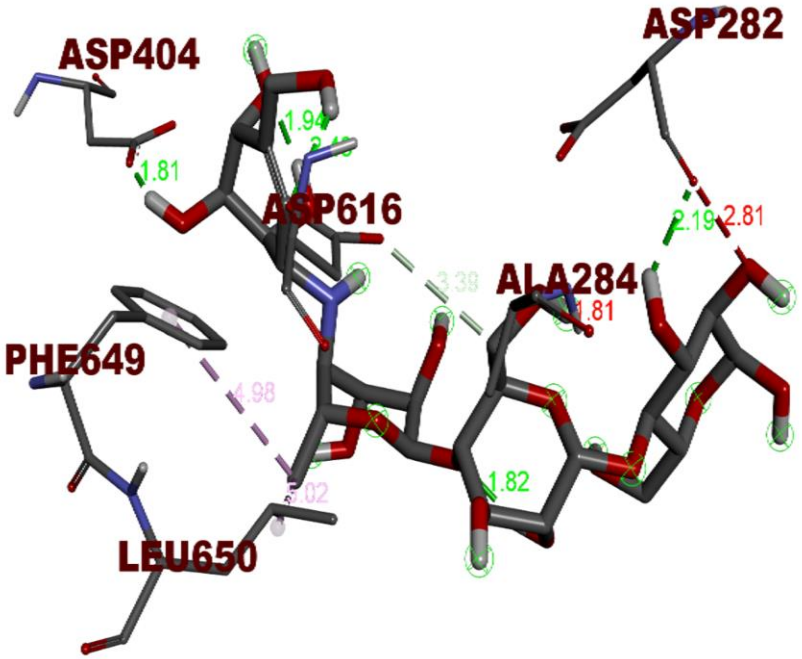


Figure 6: 2D and 3D interactions of Acarbose compound with alpha glucosidase

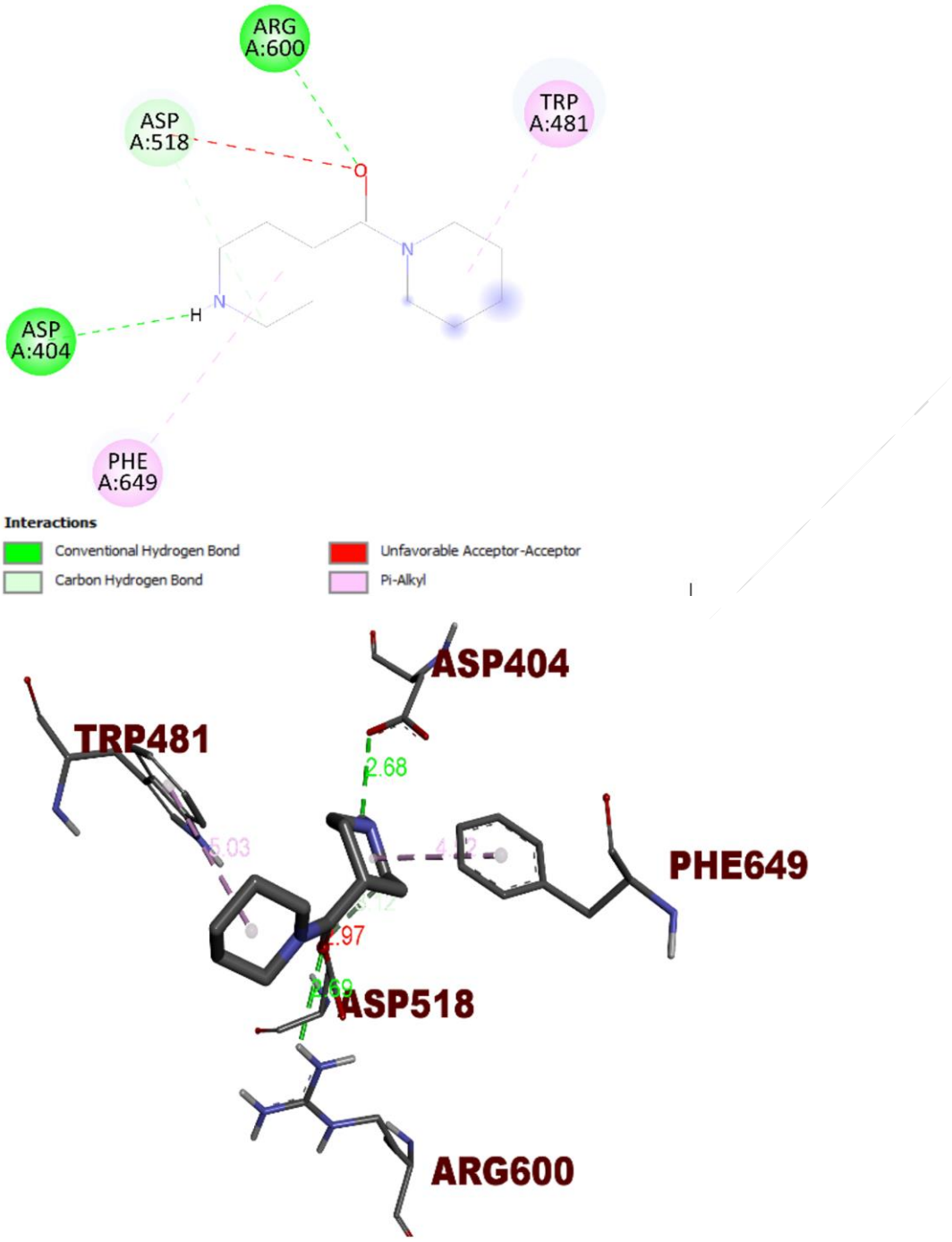


Figure 7: 2D and 3D interactions of piperidin,1-(4- piperidinylcarbonyl) compound with alpha glucosidase

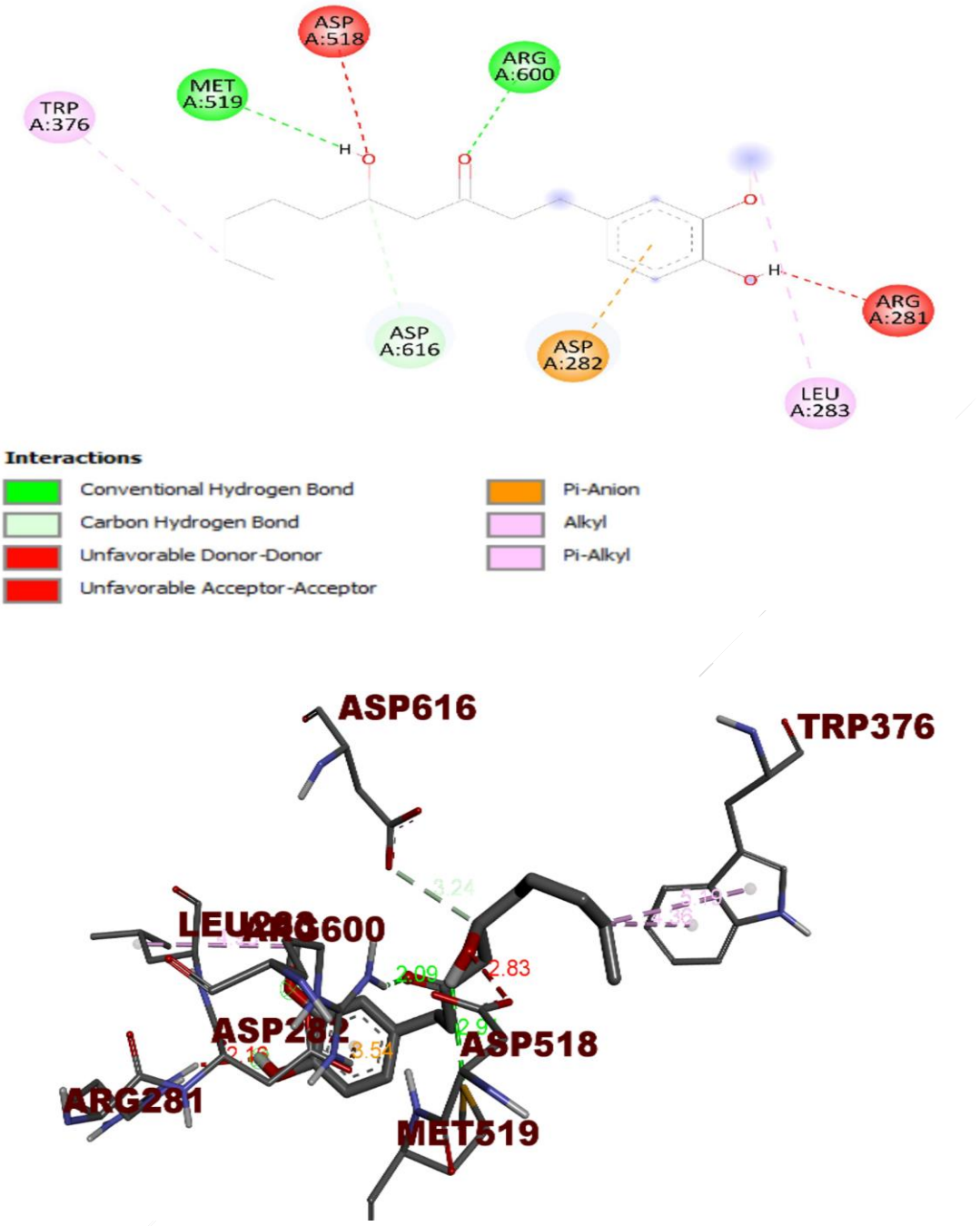


Figure 8: 2D and 3D interactions of Gingerol compound with alpha glucosidase

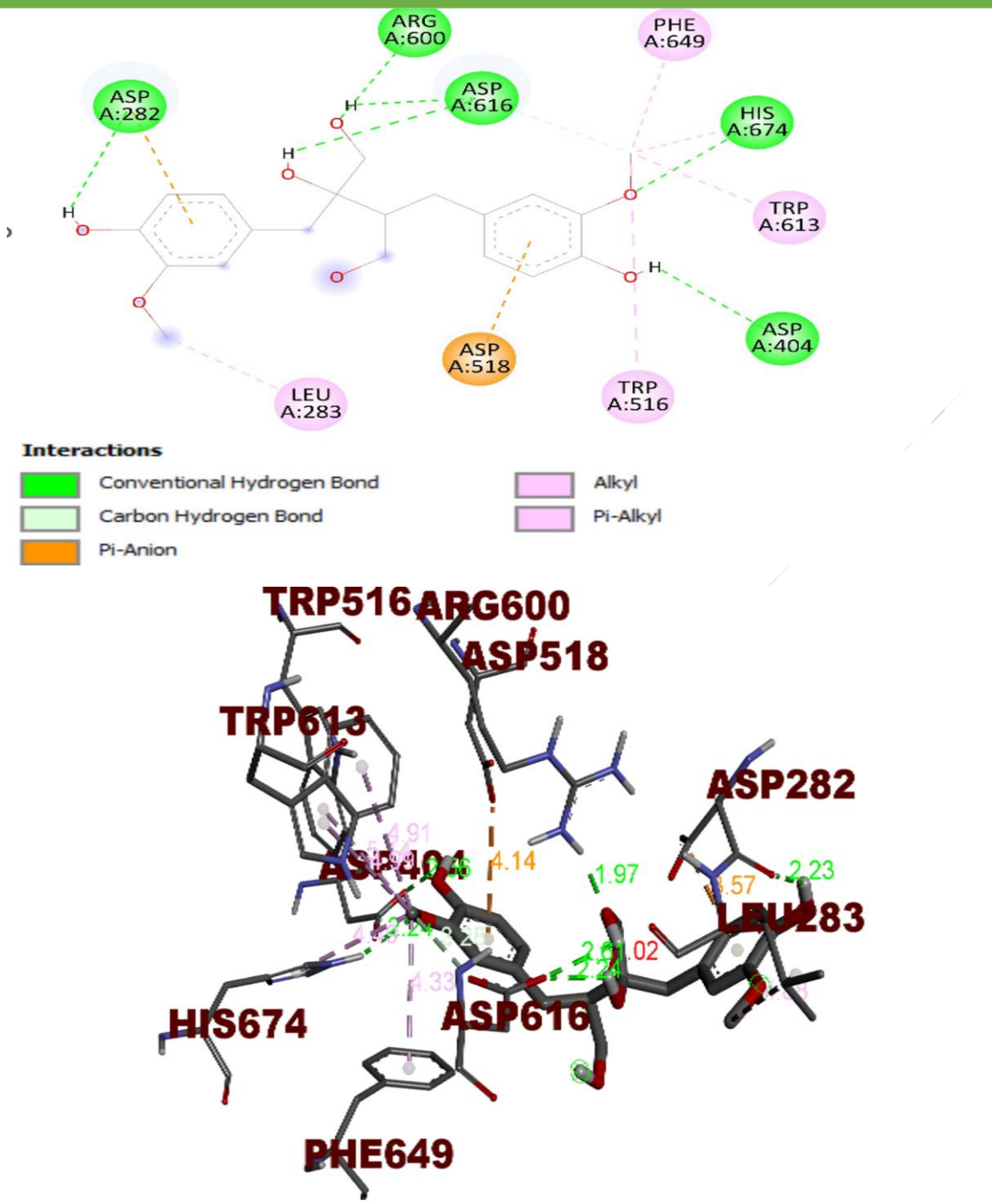


Figure 9: 2D and 3D interactions of Carinol compound with alpha glucosidase

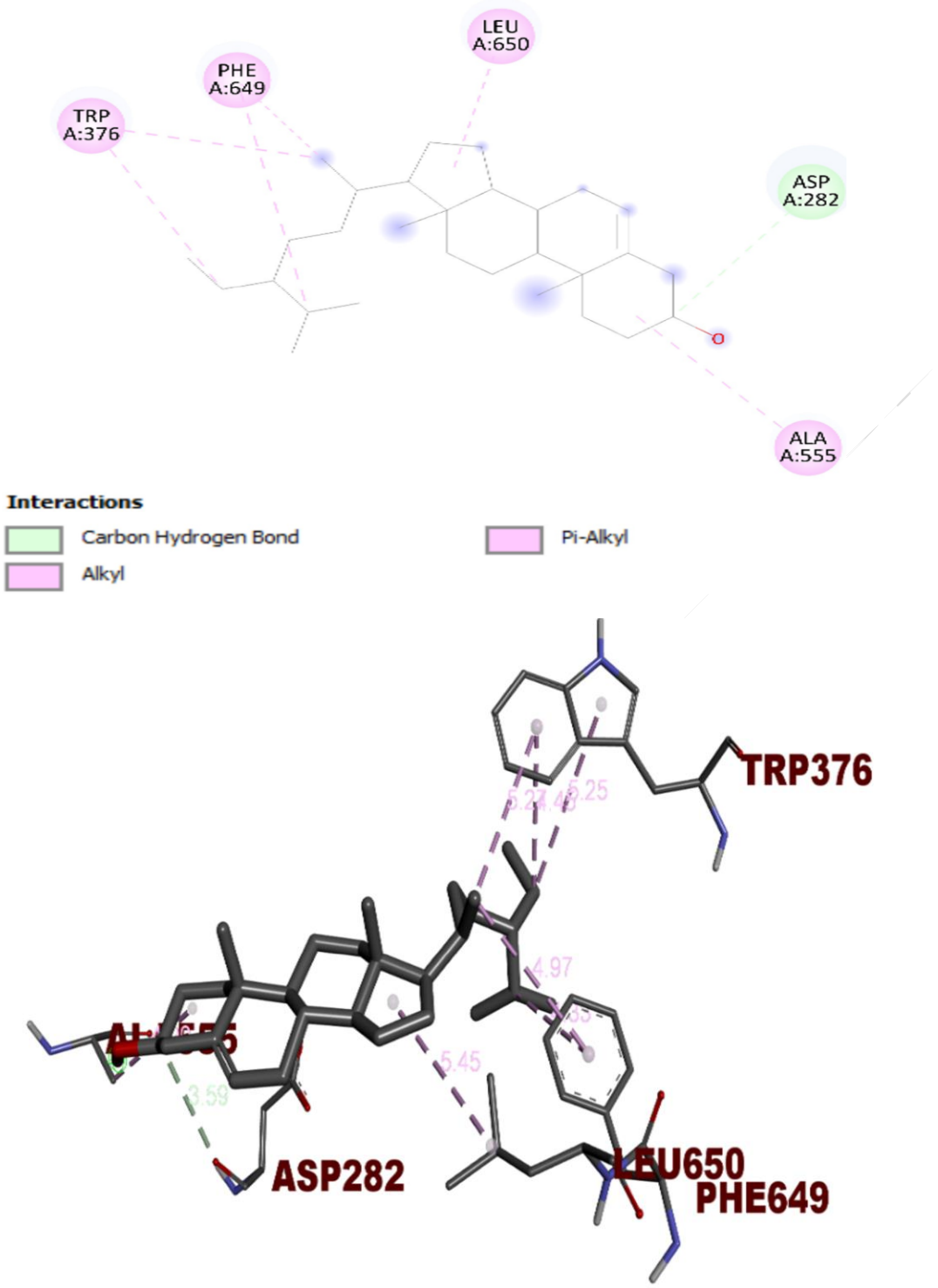


Figure 10: 2D and 3D interactions of Gamma- sitosterol compound with alpha glucosidase

LIST OF ABBREVIATIONS

CDNB: 1-chloro-2, 4-dinitrobenzene

DC: Diabetic control

DPPH: **2,2 –** Diphenyl-1-Picrylhadrazyl Radical

DTNB: 5,5′–dithiobis-2-nitrobenzoic acid

ELISA: Enzyme-linked immunosorbent assay

HOMA-IR: Homeostasis model assessment of insulin resistance

HOMA-β: Homeostasis model assessment of beta cell

Gas Chromatography Mass Spectrometry: GC-MS

GSH: Reduced glutathione

GST: Glutathione-S- transferase

GPx: Glutathione peroxidase

MDA: Malondialdehyde

MENL: Methanol extract of NL

NADPH: Nicotinamide adenine diphosphate reduced

Newbouldia laevis: NL

NC: Normal control

TBA: Thiobarbituric acid

TCA: Trichloro acetic acid