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Research Article

Anti-hyperglycemic effect of two terpenoids isolated from *Coula edulis* on normoglycemic rats and *in silico* study of their potential inhibitors on α-amylase and dipeptidylpeptidase 4

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Abstract

Diabetes causes many deaths around the world, making the search for treatments a real challenge. Plant secondary metabolites are promising candidates because they act as scaffolds in biological processes. This study investigates anti-hyperglycaemic effect of two terpenoids isolated from *Coula edulis* and *in silico* study of their potential inhibitors on α-amylase and dipeptidypeptidase 4. After extraction and isolation of the two terpenoids, their structures were characterized using 1D and 2D NMR spectroscopic techniques. Subsequently the anti-hyperglycemic effect was achieved following an overload of starch on the one hand and glucose on the other hand in normoglycemic rats. Each isolated terpenoids was tested at a dose of 3 mg/kg.bw, the same for the reference compounds (Acarbose and glibenclamide). Conformational site analysis and docking parameters such as binding energy, inhibition constant, interaction profiles with diabetes target residues (α-amylase and dipeptidypeptidase 4) were determined using AutoDock 4.2 and Discovery Studio visualizer. The results showed that the terpenoids isolated from *coula edulis* were Taraxerol and 3*β-(Z)*-coumaroyltaraxerol, each of its two terpenoids considerably decreased the blood sugar levels in rats after overloading of starch and glucose solutions respectively. Their effects were similar to the reference drugs. Furthermore, the *in-silico* approach showed that these compounds have good docking scores with α-amylase and with DPP4. Taraxerol exhibited a docking score more than three times than the acarbose docking score. Only 3*β-(Z)*-coumaroyltaraxerol reacts with at least one amino acid of the α-amylase catalytic triad (Asp 300). Both interact with histidine (His 740) of the DPP4 catalytic triad. In view of this results, taraxerol and 3*β-(Z)*-coumaroyltaraxerol have anti-hyperglycemic effects and are good candidates for the development of new multitarget antidiabetics.

Keywords: *Coula edulis*, RMN 1D and 2D, tarxerol, 3*β-(Z)*-coumaroyltaraxerol and Anti-hyperglycemia

1. INTRODUCTION

According to the International Diabetes Federation, 463 million persons suffer from diabetes worldwide, leading to 1.6 million deaths each year. This number is expected to increase by 51% to reach 700 million persons affected by 2045 [1]. Diabetes mellitus (DM) is a chronic metabolic and endocrine disorders that occurs due to defective insulin secretion by pancreatic β-cells or impaired sensitivity to insulin secreted by the pancreas, leading to chronic hyperglycaemia [2]. Hyperglycaemia contains two components: postprandial hyperglycaemia (PHG) and fasting hyperglycaemia. PHG is a phase characterized by a strong, rapid and permanent increase in blood sugar following a meal, resulting from poor control of postprandial blood sugar. It plays an important role in the development of type 2 diabetes (T2DM) and its micro and macrovascular complications. Therefore, controlling postprandial blood sugar is as important as controlling fasting blood sugar in diabetes [3]. PHG appears to

be a key factor in the management of T2DM [4]. One of the principal approaches for reducing postprandial hyperglycemia in patients with DM is the prevention of hydrolysis and the absorption of carbohydrates after food uptake. This made it possible to highlight several therapeutic targets for the treatment of T2DM, in particular enzymes digesting sugars at the intestinal level (such like α-amylase and invertase) and the DPP4 protein (which hydrolyzes the incretin hormones) [5].

alpha-amylase (α1, 4-glucan 4-glucanohydrolase, EC 3.2.1.1) is a key enzyme produced by human salivary glands and the pancreas (reverse in the intestinal lumen); It acts at random positions by hydrolyzing the α-1,4-glycosidic bonds of carbohydrates via its catalytic triad (Asp 197, Glu 233 and Asp 300). Several inhibitors of this enzyme are available as medications, namely acarbose, miglitol, voglibose and biguanides [8]. Dipeptidypeptidase 4 (DPP4; EC3.4.14.5) is a type II transmembrane protein, it is a serine exopeptidase which has a catalytic triad (Ser 630, Asp 708, His 740), which cleaves incretins such as glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide or glucose-dependent insulinotropic peptide (GIP) [9]. Drugs like Sitagliptin and vildagliptin are known as inhibitors of this protein [10]. Regardless of their varying effects, they all have a single target, they can cause side effects such as increased body weight, gastrointestinal problems, heart failure, headache, nasopharyngitis, acute respiratory infections, flatulence and do not reduce the incidence of the disease [8].

These open up a challenge for the research and identification of new molecules capable of managing postprandial hyperglycaemia by acting on several targets. With this in mind, numerous researches have shown the potential of medicinal plants to modulate this postprandial hyperglycemia [11]. Terpenoids have biological activities and may be a good candidates for the development and search for new drugs against postprandial hyperglycaemia [12]. The *in silico* approach via molecular docking is a conceptual simulation method to characterize the binding affinity of ligands to the catalytic site of receptors [13]. This approach has an important role in medicinal chemistry because it provides a good framework for the prediction of the activities of a compound (ligands) in relation to a target while comparing its scores to those of the scores of reference compounds. Recently the work of Beyegue *et al* [14] showed the inhibitory potential of digestive enzymes of stem bark extracts of *Coula edulis Baill* (*Olacaceae*), a plant in the Cameroonian pharmacopoeia occupying a place of choice in traditional medicine [15]. This potential is due to the bioactive compound it contains. In this work, we evaluated the anti-hyperglycemic effect of two terpenoids isolated from *Coula edulis* on normoglycemic rats and *in silico* study of their potential inhibitors on α-amylase and dipeptidypeptidase 4.

2. MATERIAL AND METHODS

2.1. General experimental procedure for phytochemistry

For this work, methanol and dichloromethane were used for the extraction of the plant material. n-hexane, dichloromethane, ethyl acetate, and methanol were used as pure or binary mixtures at different polarities for the purification of compounds. Column chromatography (CC) were carried out on silica gel 230-400 mesh, Merck (*Merck, Darmstadt*, Germany), 70-230 mesh (*Merck*) or Sephadex LH-20 (*Sigma-Aldrich*). Thin-layer chromatography was performed on Merck pre-coated silica gel (60 F254) aluminium foil (*Merck*) and compounds spots were detected by spraying with diluted sulfuric acid 25 % before heating the plate at about 100 °C or by visual inspection under UV lamp at 254 nm and 365 nm. The 1D and 2D NMR spectra were recorded on *Bruker Bio Spin GmbH* in deuterated solvents. Chemical shifts were reported in δ (ppm) using tetramethylsilane (TMS) (*Sigma-Aldrich*) as an internal standard, while coupling constants (J) were measured in Hz.

2.2. Plant material

For this study, the leaves of *C. edulis* were collected in *Mouko* in the Centre Region of Cameroon, in June 2018 and identified by a botanist at the National Herbarium of Cameroon (NHC), where a voucher specimen is deposited under the reference number N° 55106 HNC.

2.3. Extraction and isolation

one thousand (1000) g of dried leaves was weighed and soaked at room temperature (25 ± 2 °C) in 10 L of a mixture of dichloromethane/methanol (1:1, v/v) in a closed vessel for 72 hours; followed by filtration and evaporated to afford 112.0 g of crude extract. A portion of 108.0 g of crude extract was subjected to vacuum liquid chromatography (VLC) over silica gel and eluted with a mixture of n-hexane /EtOAc and EtOAc/MeOH gradients. Twenty (20) fractions of 500 mL each were collected and combined according to Thin layer chromatography (TLC) profiles monitoring to five fractions (F1 to F5). Fraction F2 (30.3 g) was also subjected to CC over silica gel and eluted with a mixture of n-hexane//EtOAc and EtOAc/MeOH of gradients to afford three sub-fractions F2S1 to F2S3. Successive CC on sub-fraction F2S1 (5.2 g) eluted with a mixture of n-hexane/EtOAc gradient yield compounds 9 (13.0 mg) obtained as a white powder in a mixture of n-hexane/ethyl acetate (2/8), 10 (11.7 mg) obtained as a white powder in a mixture of n-hexane/ethyl acetate (8/2), Successive CC on sub-fraction F2S2 (6.5 g) eluted with a mixture of n-hexane/EtOAc gradient yield compounds 11 (15.0 mg) obtained as a white powder in a mixture of n-hexane/ethyl acetate (7/3).

2.4. *In vivo* study

2.4.1. Anti-hyperglycemic potential of terpenoids on postprandial glucose in normoglycemic rats

2.4.1.1. Animals and food

The anti-hyperglycemic effects of the two terpenoids (taraxerol and 3*β-(Z)*-coumaroyltaraxerol) were carried out on twenty-five (25) male Wistar strain rats. Thhey were provided by the Laboratory of Nutrition and Nutritional Biochemistry (LNNB) of the Biochemistry Department, Faculty of Sciences, University of Yaoundé I. The animals were kept at room temperature (25 ± 1 °C), subjected to a day-night cycle of 12h (7h-19h day and 19h-7h night) in good conditions of ventilation and natural lighting. They had *ad libidum* access to running tap water and a conventional diet for breeding (normal diet, ND) rodents. None of these animals was subjected to previous experiments and showed signs of abnormalities. The animals were introduced in standard cages with the respect of the conditions of hygiene and ethics for animals, with the guidelines of the ethics committee of the University of Yaoundé 1 and the Guide for the care and use of laboratory animals (8th edition)

2.4.1.2. Evaluation of the effects of the two terpenoids on glucose tolerance in normal male rats

One day before handling, the rats were fasted for a period of 12h. At the beginning of the experiment, the rats were weighed using an electronic scale. Blood glucose level was assessed using glucose test strips and a glucometer (*OneTouch Ultra-Easy*) by collecting blood from the caudal tail using a sterile lancet. Subsequently, the rats were divided into 5 groups (five rats each) depending on the average blood glucose level (mg/dL) as follows

Negative control (NC) group: receiving water

Positive control group (PC): receiving water + glucose solution at dose of 2 g/kg.Bw

Reference group (Ref): receiving glibenclamide (3 mg/kg.Bw) + glucose solution at dose of 2 g/kg.Bw

Assay group 1: receiving Taraxerol (3 mg/kg.Bw) + glucose solution at dose of 2 g/kg.Bw

Assay group 2: receiving 3*β-(Z)*-coumaroyltaraxerol (3 mg/kg.Bw) + glucose solution at dose of 2 g/kg.Bw

At the initial time (t_0), the negative control (NC) and positive control (PC) groups received water by esophageal gavage while the reference and test groups received respectively glibenclamide, and the two terpenoid solutions. Thirty minutes after administration of these different solutions, the glucose overload solution was administered to all groups except the NC group; the blood glucose levels were measured at 30, 90, 150 and 240 min respectively and the results recorded.

2.4.1.3. Evaluation of the effects of the two terpenoids on the inhibition of alpha-amylase in normal male rats

The inhibition of alpha-amylase by the two terpenoids was evaluated by assessing the digestibility of starch in rats. For this experiment, the protocol used above for the glucose tolerance test (OGTT) with slights modifications. This modifications were the replacement of glucose by starch gelatinized at the dose of 2 g/kg.Bw. Acarbose at the dose of 3 mg/kg.Bw was used as reference drug. As in the case of OGTT, rats received water (NC and PC), Acarbose (Reference), and Assay at a dose of 3 mg/kg.Bw. After 30 min, starch gelatinized (2 g/kg.Bw) solution was administered to all the groups except the NC group. Subsequently (30 min thereafter), the blood glucose levels were measured 90, 150 and 240 min using impregnated strips and a *OneTouch Ultra* brand blood glucometer by stinging at the rat's tail and the results were noted.

2.5. *In silico* study using a Molecular docking approach

2.5.1. Software used

The following software's *Python* 2.5, and 2.7, *Molecular Graphics Lab Tools (MGL)*, *AutoDockTools*-1.5.7, *Discovery Studio Visualizer* 2.5.5, and *ChemDraw* were used. The *Python* 2.7 was downloaded from www.python.com, Cygwin (a data store) c:\program; and *Python* 2.5 were downloaded simultaneously from www.cygwin.com. *Molecular Graphics Lab Tools* (MGL) and AutoDockTools-1.5.7 were downloaded from www.scripps.edu; *Discovery Studio Visualizer* 2.5.5 was downloaded from www.accelerys.com; and *ChemDraw* was downloaded from www.clubic.com. Online smile scoring was performed using cactus.nci.nih.gov/translate/ [16].

2.5.2. Preparation of the ligand

Two-dimensional structures (2D) of the taraxerol and *3β-(Z)*-coumaroyltaraxerol and drug reference (*Acarbose* and *Sitagliptin*) compounds were drawn using *ChemDraw* software. Three-dimensional structures (3D) were obtained using *chemdraw* 3D software and energy minimization was performed using the Molecular Mechanics (MM2) force field and Saved in PDB format with the same software

2.5.3. Preparation of the target enzyme

The crystal structure of α-amylase protein (ID: 1B2Y) and DPP4 (ID: 1R9N) was downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) protein database. The preparation of the target proteins with the *AutoDock* tools involved the addition of all hydrogen atoms to the macromolecule, a necessary step for the correct calculation of partial atomic charges. Three-dimensional affinity grids of α-amylase contained the amino acids of its active site [Asp 197, Glu 233, and Asp 300 [17]] of size 40 × 40 × 44 Å with spacing of 0.375 Å, the macromolecule with Y and Z coordinates of 8.22, 47.41 and 19.66 . Three-dimensional affinity grids of DDP4 contained the Catalytic Triad [Ser 630, Asp 708, His 740 [9]] of size 40 × 40 × 40 Å with spacing of 0.375 Å, the macromolecule with X, Y and Z coordinates of 24.173, 0.944 and 16.655.

2.5.4. Docking Simulations

The molecular docking used the *Lamarckian* genetic algorithm method, which performed a total of 2,500,000 energy calculations for every run and 10 total generations. The algorithm method works using traditional force fields, providing empirical free energy functions and a binding energy constant (μM) with "master equation" as follows:

$$\Delta G = \Delta G_{vdw} + \Delta G_{hbond} + \Delta G_{elec} + \Delta G_{conform} + \Delta G_{tor} + \Delta G_{sol}$$

The typical molecular mechanics terms are the van der Waals interaction (ΔGvdw + desolv), electrostatic interaction (ΔGelec), torsional energy (ΔGtor), desolvation upon binding, and the hydrophobic effect (+ΔGsol) [18]. The other docking simulations were carried out with *AutoDock* default parameters. *Discovery Studio Visualizer* software was used to visualize the protein–ligand interactions in 3D and 2D.

2.6. Statistical analysis

The *R commander* software version 4.2.1 was used and one-way analysis of variance (ANOVA) with the Tukey test was performed to compare variability between groups. Significant differences were detected at a 95% confidence interval and the results obtained were expressed as mean ± standard deviation.

3. RESULTS

3.1. Physical and spectroscopic data of the compounds 1 and 2

Compound 1, taraxerol: white powder (65 mg); mp: 281-283 °C ; MS (m/z): 426.3917 [M]+ $C_{30}H_{50}O$; IR v max (CCl_4) cm-1:3486, 2932, 2854, 1471, 1444, 1381, 1035;1H NMR (500 MHz, CDCl3) δ 5.56 (dd, J = 8.1, 3.3 Hz, 1H-15), 3.22 (dd, J = 11.7, 3.6 Hz, H-3), 2.06 (dt, J = 12.8, 3.1 Hz, 1H), 1.94 (dd, J = 14.7, 3.2 Hz, H-16), 0.90 (s, Me-23 et Me-24), 1.08 (s Me-25) 1.12 (s, Me-26 et Me-27) 0.75(s,Me-28), 0.92 (s, Me-29 et Me-30).13C NMR (125 MHz, CDCl3):28.0 (C-23)15.4 (C-24), 15.4 (C-25), 29.9 (C-26), 25.8 (C-27), 29.9 (C-28), 33.3 (C-29), 21.3 (C-30).

Compound 2, *3β-(Z)*-coumaroyltaraxerol: white powder (5.1 mg); mp: 260-262 °C; MS (m/z): 572.4138[M]+ $C_{39}H_{57}O$; IR v max (CCl_4) cm-1:3383, 2927, 2856, 1707, 1602, 1510, 1450, 1377, 1163 1H NMR (500 MHz, DMSO-d6) δ 4.42 (t, J = 8.0 Hz, H-3), 5.48 (dd, J = 8.2, 3.1 Hz, H-15),5.71 (dd, J = 12.8, 1.1 Hz, H-2'), 6.77 (d, J = 12.8 Hz, H-3'), 7.59 (d, J = 8.2 Hz, H-5), 6.71 (m, H-6), 6.71 (m, H-8'),7.59 (d, J = 8.2 Hz, H-9), 9.71 (s, OH), 13C NMR (125 MHz, DMSO-d6) δ 80.4 (C-3), 157.9 (C-14), 116.8 (C-15), 166.5 (C-1'), 116.4 (C-2'), 143.3 (C-3'), 126 (C-4'), 132.7 (C-5'), 115.2 (C-6'), 159.0 (C-7'),115.2 (C-8'), 132.7 (C-9').

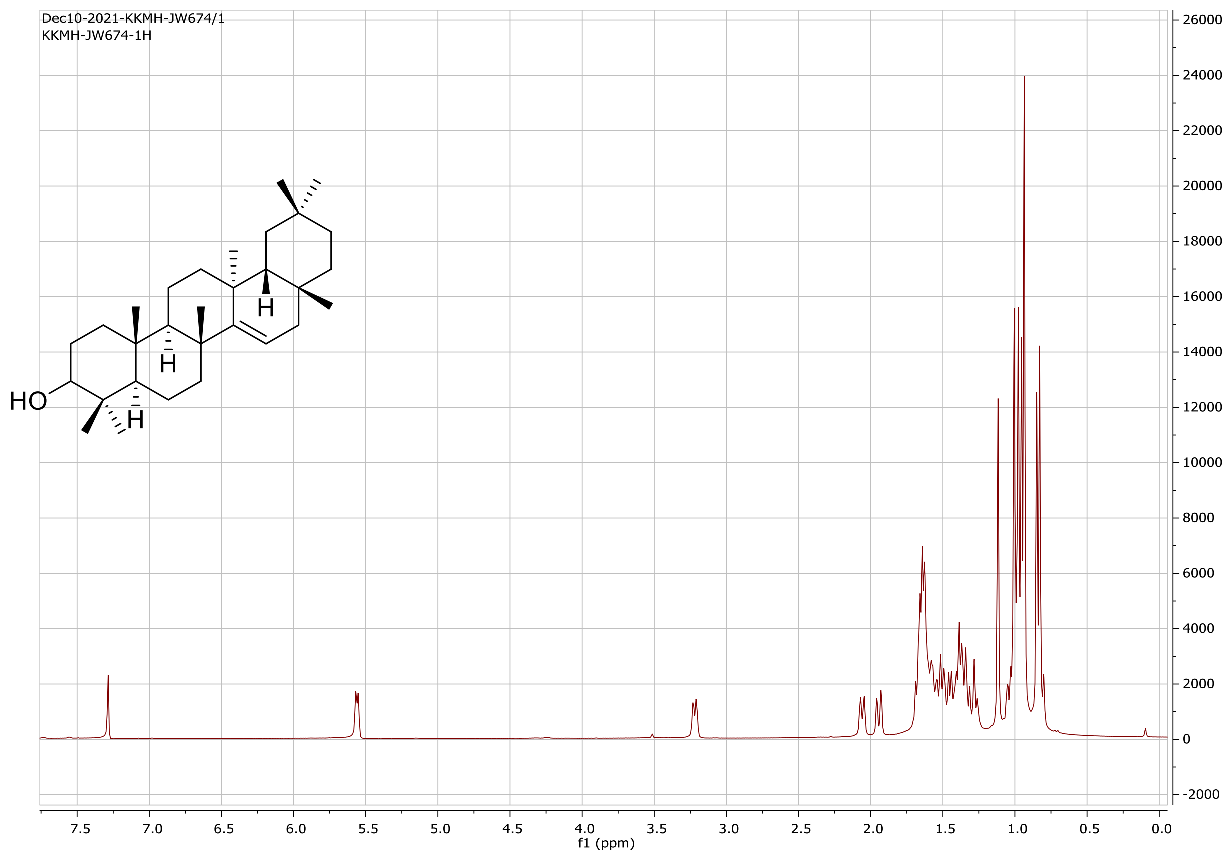

Figure 1a: ^{1}H NMR spectrum of taraxerol (1) ($CDCl_3$, 500 MHz)

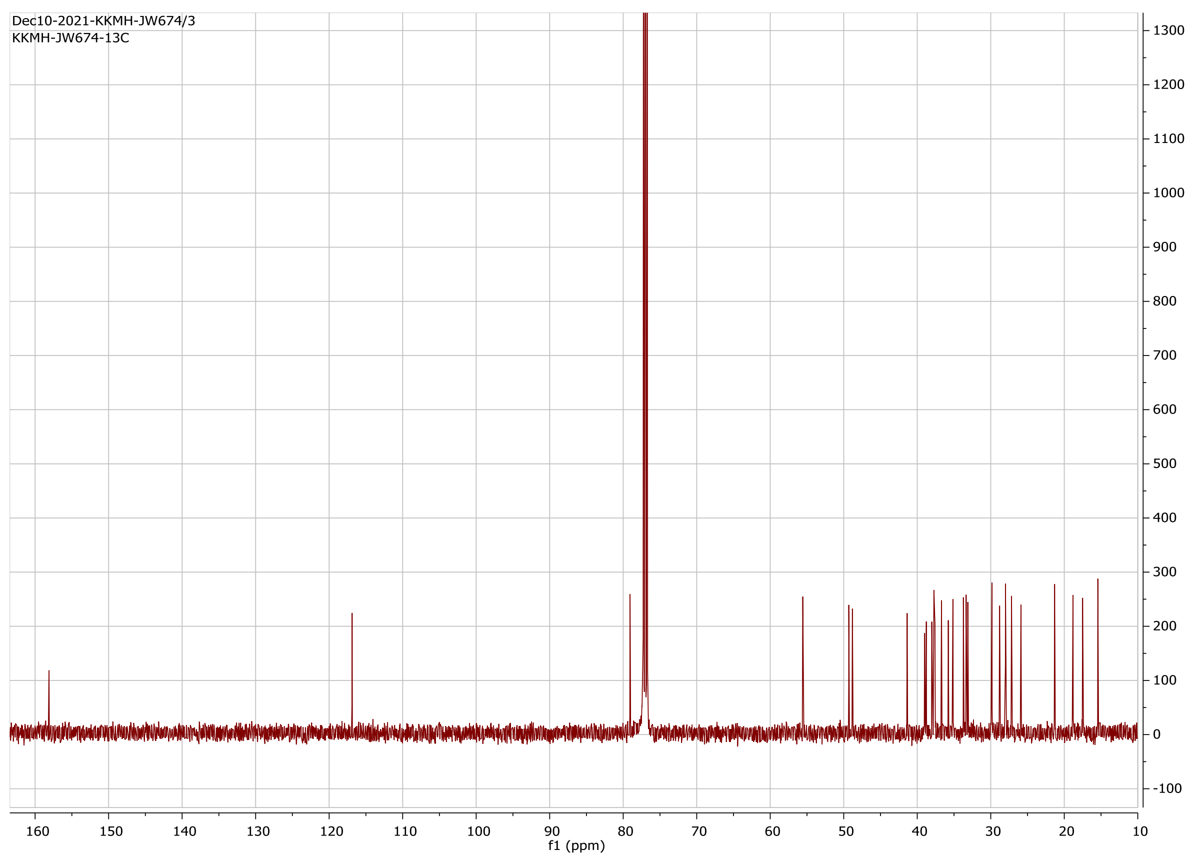

Figure 1b: ^{13}C NMR spectrum of taraxerol (1)

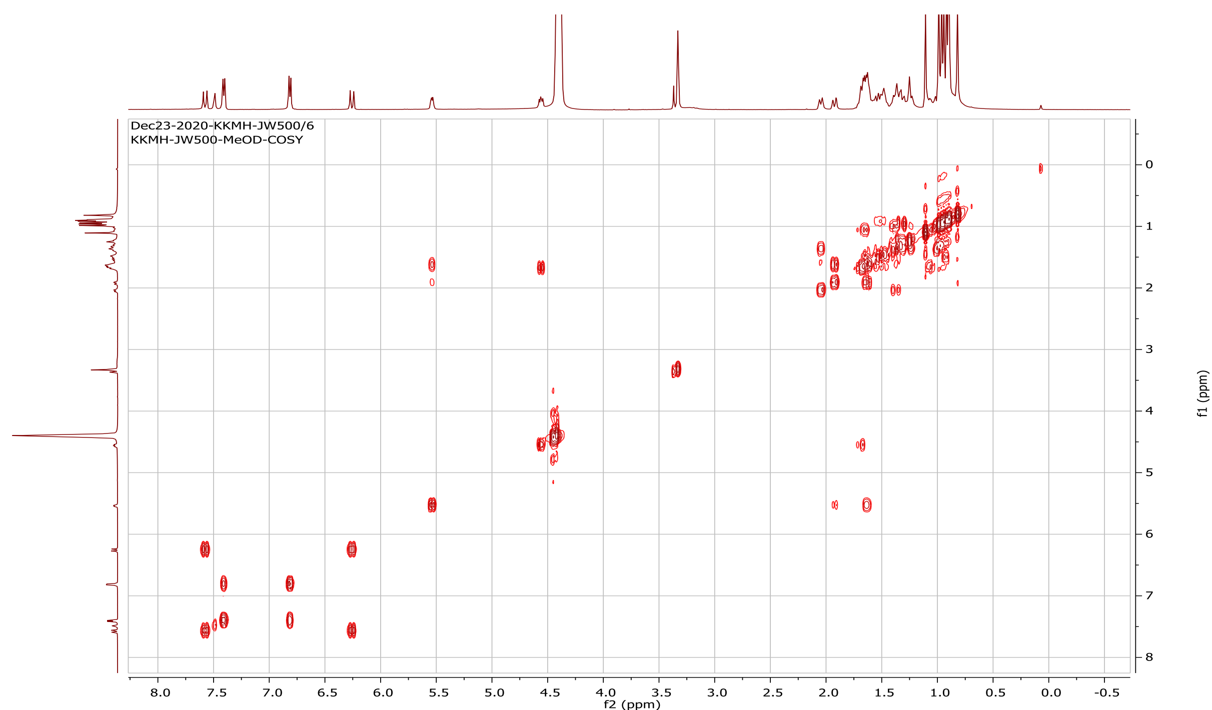

Figure 2a: COSY spectrum of 3β-(Z)-coumaroyltaraxerol (2) ($CDCl_3$)

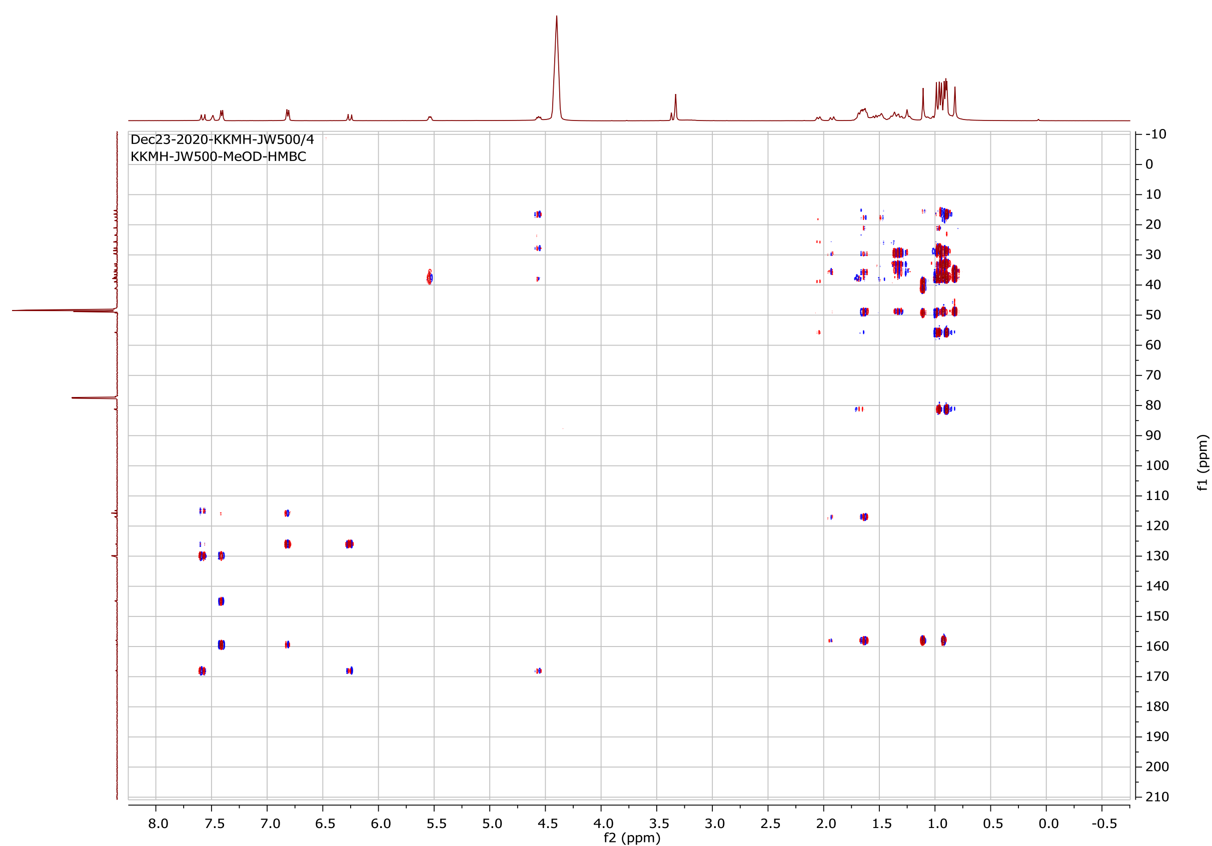

Figure 2b: ^{13}C NMR spectrum of 3β-(Z)-coumaroyltaraxerol (2) ($CDCl_3$, 125 MHz)

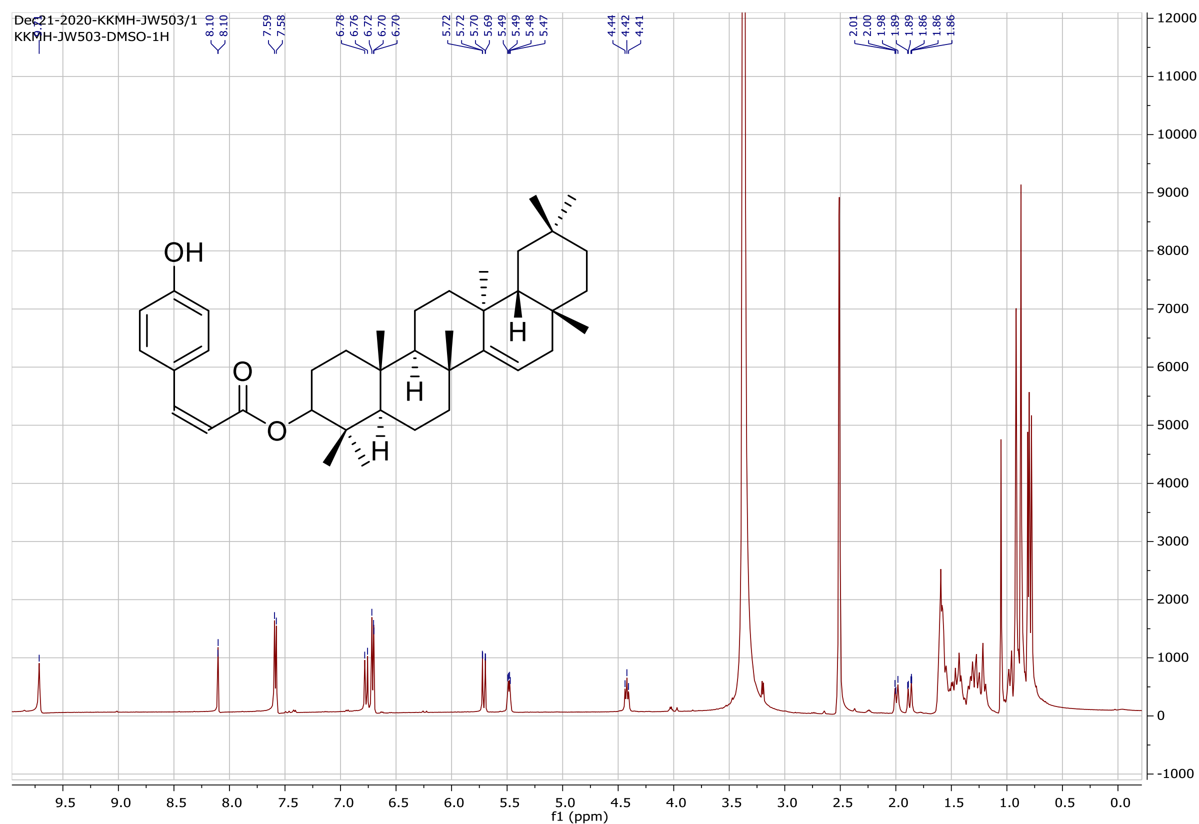

Figure 2c: ^{1}H NMR spectrum of 3β-(Z)-coumaroyltaraxerol (2) ($CDCl_3$, 500 MHz)

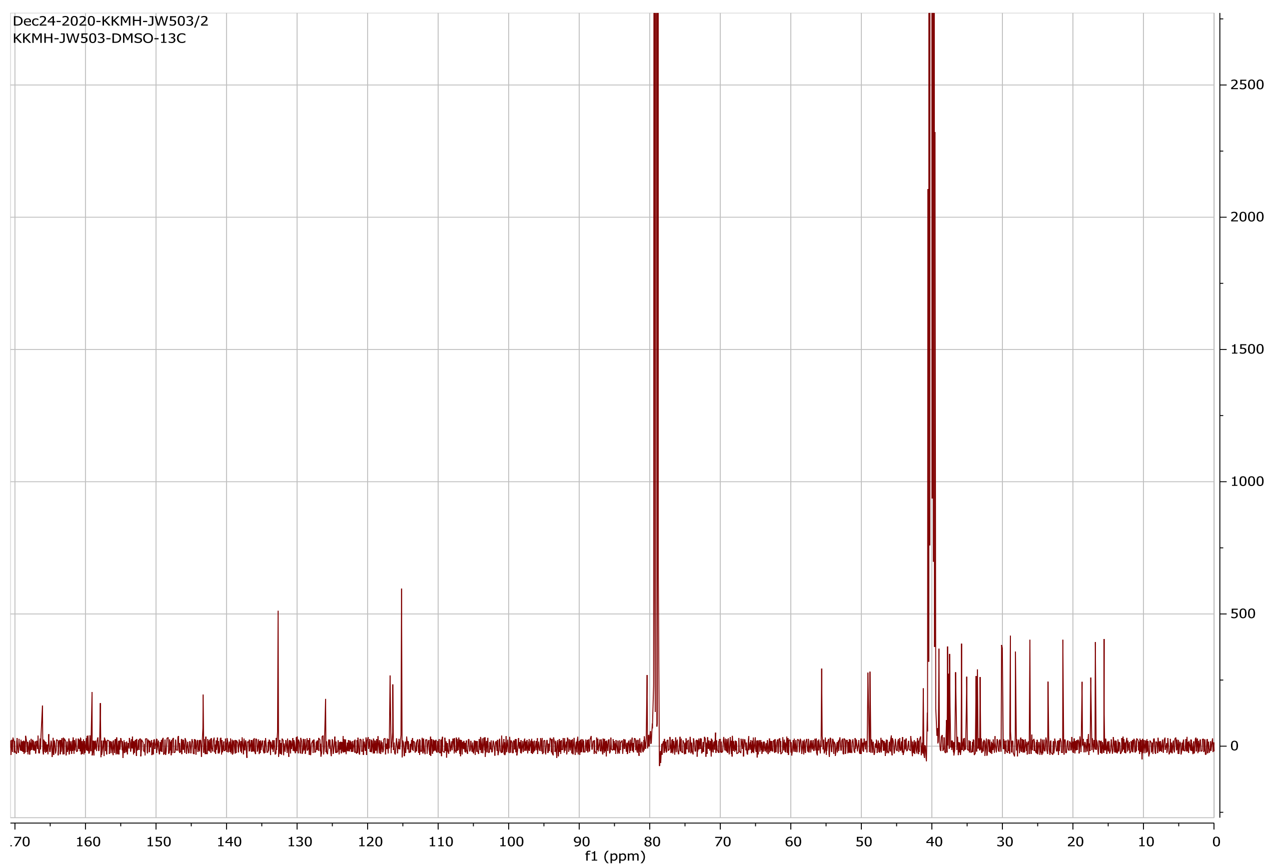

Figure 2d: ^{13}C NMR spectrum of 3β-(Z)-coumaroyltaraxerol (2) ($CDCl_3$, 125 MHz)

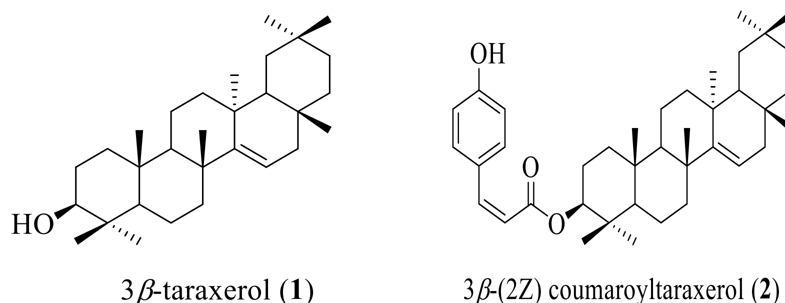

Figure 2e: Structures of the two isolated terpenoids

3.2. Anti-hyperglycemic potential of the two terpenoids in normoglycemic rats

3.2.1. Effect of the two terpenoids on blood glucose level after glucose overload in normoglycemic rats

The evolution of blood glucose level after glucose administration is presented in Table 1.

Table 1: Effect of compounds on glycaemia's.

	0 min	30 min	90 min	150 min	240 min
NC (5 mL/kg . Bw distilled water)	90,67 ± 2.04[a]	92,89 ± 3.45 [a] **(2,38%)**	92.43±9.34 [a] **(1,9%)**	89,87 ± 9.29 [a] **(-0,89%)**	99,33±7,03 [a] **(8,71%)**
PC (2 g/kg. Bw glucose + 5 mL/kg. Bw distilled water)	93,01± 4,61 [a]	167,12±9,9 [b#] **(44,34 %)**	158,33±4,94 [b #] **(41,25%)**	149,07±62 [b#] **(37,6 %)**	118,98 ±7,92 [b#] **(21,82%)**
Assay 1 (2 g/kg. Bw glucose + 3 mg/kg. Bw Taraxerol)	93,9± 2,9 [a]	143,17±2,32 [c#] **(34,41 %)**	139,43±8,53 [c #] **(32,65%)**	121,51±7,19 [c#] **(22,72%)**	109,11±9,73 [b] **(13,94 %)**
Assay 2 (2 g/kg. Bw glucose + 3 mg/kg. Bw 3*β-(Z)*-coumaroyltaraxerol)	98,12± 7,3 [a]	152,09±6,38 [d#] **(35,48 %)**	147,93±8,27 [c#] **(33,67%)**	140,4±3,94 [b#] **(30,11%)**	121,16±9,94 [c#] **(19,01%)**
Ref (2 g/kg. Bw glucose + 3 mg/kg. Bw glibenglamide)	95,87±5,01 [a]	148,15±9,27 [c#] **(35,28%)**	115,5±12,09 [d #] **(16,99%)**	99,71±5,9 [a] **(3,85%)**	89,83±4,28 [a] **(-6,72%)**

Values in parentheses are percent change; the results are expressed as mean blood glucose ± standard deviation. The values assigned to the different letters are significantly different at $p<0.05$ between the different groups at the same time. # ($p<0.05$): significant difference in the same group compared to t_0

The positive control (PC) group expressed the highest glycaemic values compared to the negative control (NC) group throughout the experiment. The tested compounds significantly ($P < 0.05$) limited the blood sugar peak. Thereafter, a significant and progressive decrease of blood glucose level was observed in the tested groups up to 240 minutes. The compounds (Taraxerol and 3*β-(Z)*-coumaroyltaraxerol) limit the increase in blood sugar in the same way as the reference drug glibenclamide.

3.2.2. Effect of the two terpenoids on blood glucose after starch overload in normoglycemic rats

The evolution of blood glucose after starch administration is presented in the Table 2 following.

Table 2: Effect of compounds on starch digestion.

	0 min	**30 min**	**90 min**	**150 min**	**240 min**
NC (5 mL/kg . Bw distilled water)	90,67 ± 5.04[a]	92,89 ± 3.45 [a] **(2,38%)**	92.43±9.34 [a] **(1,9%)**	89,87 ± 9.29 [a] **(-0,89%)**	99,33±7,03 [a] **(8,71%)**
PC (2 g/kg. Bw starch + 5 mL/kg bw distilled water)	97,01± 7.64 [a]	120, 34±7,27[b#] **(19,16%)**	143, 83±19,70 [b#] **(32.16%)**	116,2±5,32 [b#] **(-16,37%)**	96 ±3,81 [a] **(-1,04%)**
Assay 1 (2 g/kg.Bw starch + 3 mg/kg. Bw taraxerol)	83,9± 5,12 [a]	91,67±9,07 [a] **(8,47%)**	82,1±21,38 [a] **(-2,19%)**	71,09±14,73 [a] **(-18,01%)**	88,67±10,12 [a] **(-5,37%)**
Assay 2 (2 g/kg. Bw starch + 3 mg/kg. Bw 3*β-(Z)*-coumaroyltaraxerol)	94,12± 6,23 [a]	99,67±8,50 [a] **(5,56%)**	74,33±7,51 [c#] **(-26,62%)**	75,67±5,12 [c#] **(-25,38%)**	84,6±4,52 [c#] **(-11,25%)**
Ref (2 g/kg. Bw starch + 3 mg/kg. Bw Acarbose)	85,87±2,73 [a]	91,06±9,17 [a] **(5,69%)**	76,33±3,43 [c#] **(-12,49%)**	74,33±4,82 [c#] **(-15,52%)**	70,11±6,51 [c#] **(-22,47%)**

Values in parentheses are percent change; the results are expressed as mean blood glucose ± standard deviation. The values assigned to the different letters are significantly different at $p < 0.05$ between the different groups at the same time. # ($p<0.05$): significant difference in the same group compared to t_0

The positive control (PC) expressed the highest glycaemic values compared to the negative group (NC) throughout the experiment. The test compounds significantly limited ($P < 0.05$) the blood sugar peak. Subsequently, a significant and progressive decrease of blood glucose level was observed in the test groups up to 240 minutes. The compounds limited blood sugar elevations in a similar manner to Acarbose.

3.4. *In silico* inhibition of α-amylase activity

3.4.1. Molecular docking scores with taraxerol and 3*β-(Z)*-coumaroyltaraxerol

The binding affinity results of the ligands against the selected α-amylase activity target for DT2 are shown in Table 3.

Table 3: Molecular docking scores of the compound against α-amylase

Name of the Compound	Binding Energy (Kcal/mol)	Inhibition Constant (kI) (µM)
Taraxerol	-9.88	57.24
3*β-(Z)*-coumaroyltaraxerol	-8.40	693.02
Acarbose	-3.64	2160

Docking scores of -8.4 were recorded with 3*β-(Z)*-coumaroyltaraxerol and -9.88 with taraxerol. The docking score of the compounds of interest is higher than that of the reference drug (Acarbose).

3.4.2. Profiles of amino acid residues in the α-amylase interacting with taraxerol and 3*β-(Z)*-coumaroyltaraxerol

Table 4, figures 3 a, b and C show the various residues from the major sites from which the compounds of interest interact.

Table 4: Profiles of amino acid residues important for α-amylase that interact with taraxerol and 3*β-(Z)*-coumaroyltaraxerol

Compounds	Asp 197	Glu 233	Asp 300
Taraxerol	-	-	-
3*β-(Z)*-coumaroyltaraxerol	-	-	+
Acarbose	+	+	+

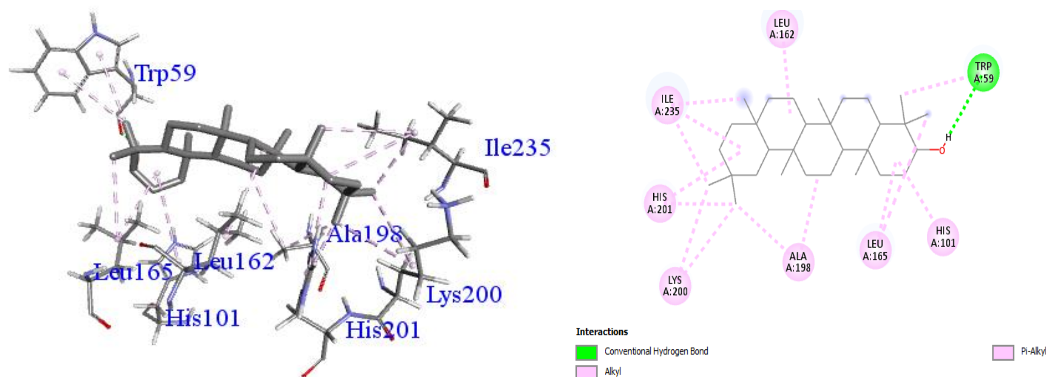

Figure 3a: 3D and 2D views of the molecular interactions between the amino acid residues of α-amylase and taraxerol

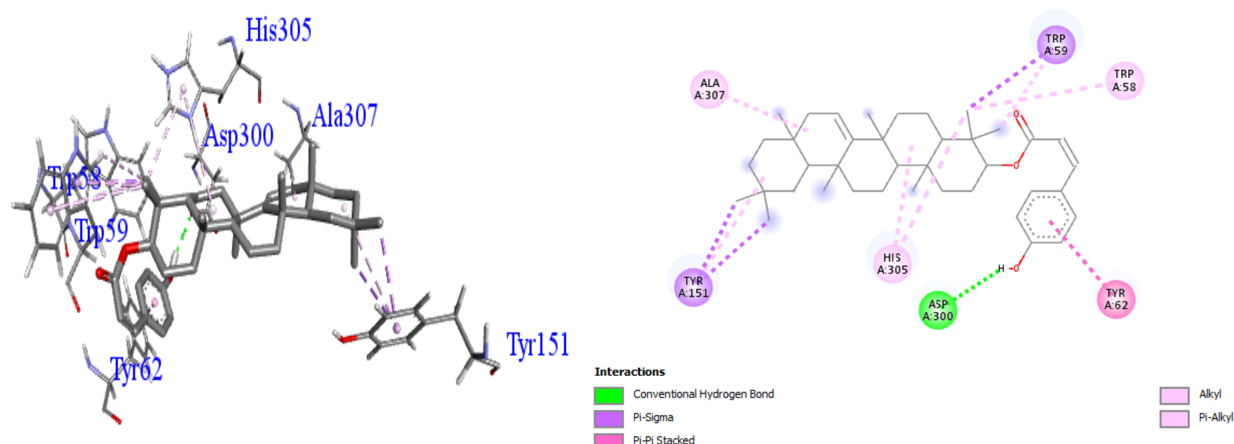

Figure 3b: 3D and 2D views of the molecular interactions between the amino acid residues of α-amylase and 3*β-(Z)*-coumaroyltaraxerol

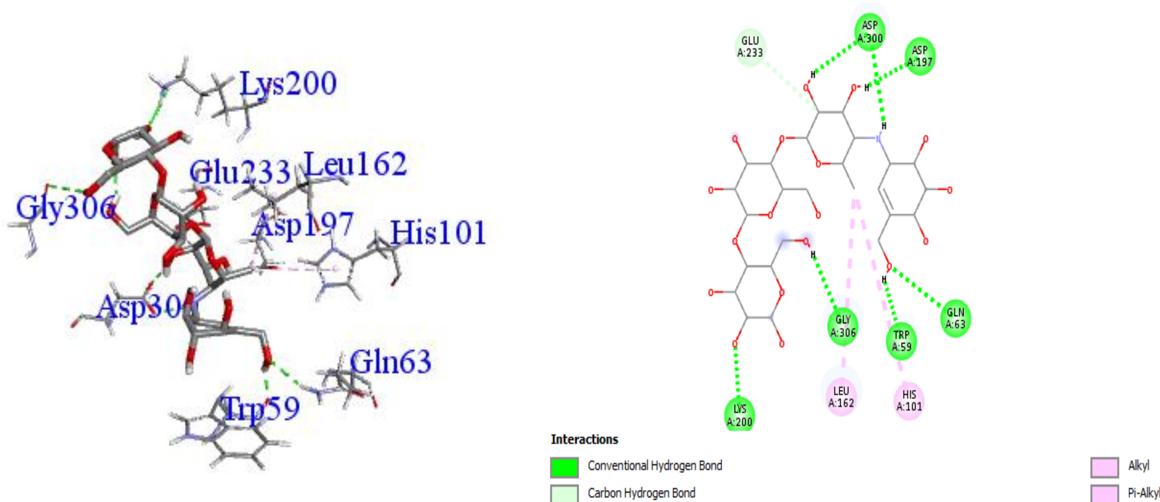

Figure 3c: 3D and 2D views of the molecular interactions between the amino acid residues of α-amylase and Acarbose

The results show that only 3*β*-*(Z)*-coumaroyltaraxerol reacts with an amino acid of the amylase catalytic triad (Asp 300). The reference drug acarbose reacts with all the amino acids of the catalytic triad.

3.5. *In silico* inhibition of DPP4 activity

3.5.1. Molecular docking scores with taraxerol and 3*β*-*(Z)*-coumaroyltaraxerol

The binding affinity results of the ligands against the selected DPP4 activity target for DT2 are shown in Table 5.

Table 5: Molecular docking scores of the compound against DPP4

Name of the Compound	**Binding Energy (**Kcal/mol**)**	**Inhibition Constant (**kI**)** (µM)
Taraxerol	-6.82	10.06
3*β*-*(Z)*-coumaroyltaraxerol	-4.80	300
Sitagliptin	-7.87	1.72

Docking scores of -4.8 were recorded with 3*β*-*(Z)*-coumaroyltaraxerol and -6.82 with taraxerol. These were slightly lower than that of the reference drug.

3.5.2. Profiles of amino acid residues in the DPP4 interacting with taraxerol and 3*β*-*(Z)*-coumaroyltaraxerol

Table 6 and figures 4 a, b and C show the various residues from the major sites from which the compounds of interest interact.

Table 6: Profiles of amino acid residues important for DPP4 that interact with taraxerol and 3*β*-*(Z)*-coumaroyltaraxerol

Compounds	Ser 630	Asp 708	His 740
taraxerol	-	-	+
3*β*-*(Z)*-coumaroyltaraxerol	-	-	+
Sitagliptin	+	-	+

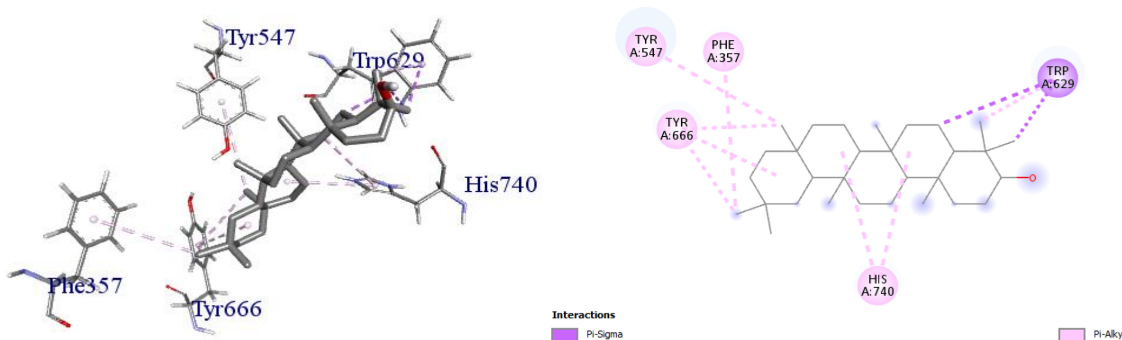

Figure 4a: 3D and 2D views of the molecular interactions between the amino acid residues of DPP4 and taraxerol

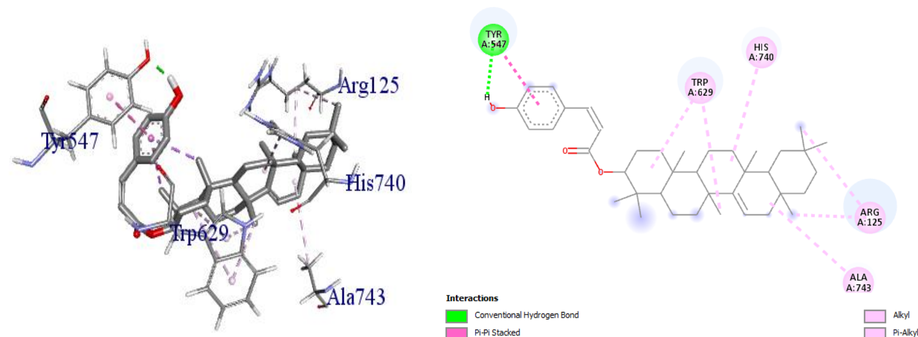

Figure 4b: 3D and 2D views of the molecular interactions between the amino acid residues of DPP4 and 3*β-(Z)*-coumaroyltaraxerol

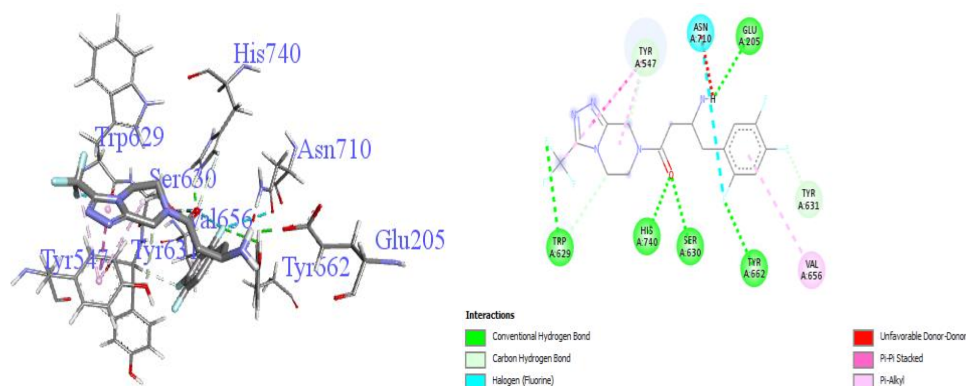

Figure 4c: 3D and 2D views of the molecular interactions between the amino acid residues of DPP4 and Sitagliptin

The results show that taraxerol and 3*β-(Z)*-coumaroyltaraxerol all react with His 740 reactive DPP4. In addition to the reactive His 740 of DPP4, the reference drug interacted with Ser 630, an important active site of the protein.

4. DISCUSSION

Diabetes is the cause of several deaths throughout the world, making the search for treatments a real challenge. numerous researches have shown the potential of medicinal plants to modulate postprandial hyperglycaemia which seems to be the ideal therapeutic target for the control of glyceamia in the management of diabetes. Study showed the inhibitory potential of digestive enzymes (α-amylase, invertase) of stem bark extracts of *Coula edulis Baill* (*Olacaceae*)[14]. The present study aimed to evaluate the antihyperglycemic effect of two terpenoids isolated from *Coula edulis* (plant with antidiabetic potential) and to carry out an *in silico* study on α-amylase and DPP4 inhibitory mechanisms.

A phytochemical study was carried out to isolate and characterize the two terpenoids of the plant. We obtained taraxerol and 3*β-(Z)*-coumaroyltaraxerol with data that corroborate those reported by [19]. Subsequently, an evaluation of the effect of its two terpenoids on the evolution of blood sugar in normoglycemic rats having received respectively an overload of starch and glucose, was carried out. To reduce postprandial hyperglycaemia, which is a component of diabetic hyperglycaemia, it would be interesting to reduce the activity of complex carbohydrate enzymes after food intake and in particular of α-amylase with the consequence of reducing glucose production and therefore its absorption [20]. The two terpenoids (taraxerol and 3*β-(Z)*-coumaroyltaraxerol) have a limited glycaemic peak in rats after administration of starch compared to the positive control. This effect was more noted with 3*β-(Z)*-coumaroyltaraxerol. This result can be explained by the fact that, the two compounds act mainly by inhibiting the activities of α-amylase and therefore by inhibiting starch digestion. *In silico* evidence showed the inhibitory potential of our two compounds on α-amylase with docking scores greater than the reference molecules. The interaction between the 3*β-(Z)*-coumaroyltaraxerol and Asp 300 of the catalytic triad reflects the fact that the latter can carry out competitive inhibition in the same way as the reference drug Acarbose. The absence of interaction between the residues of the catalytic triad of α-amylase and taraxerol may reflect the fact that the latter is a non-competitive inhibitor of this enzyme. This result explains the α-amylase inhibitory potential of *Coula edulis* extract [14]. The administration of the two terpenoids to the different test groups made it possible to considerably reduce the peak of blood glucose in normoglycemic rats which received a glucose boost. This effect was more noted with taraxerol. the reduction in the glycaemic peak observed may be due to the fact that these two compounds stimulate the production of insulin in the pancreas [21]. *In silico* evidence showed that both terpenoids bind to DPP4 with a good score and interact with the active site reactive His. This result is in the same direction as the work of Pulikkottil *et al* [22] which showed the inhibitory potential of terpenoids on DPP4. In fact, DPP4 inhibitors are used to manage hyperglycaemia in diabetics and they have no intrinsic hypoglycaemic activity [23]. Its inhibition makes it possible to increase the half-life of incretin hormones and to reduce blood sugar which will make it possible to better manage T2DM.

5. CONCLUSION

This study showed that both terpenoids (taraxerol and 3*β-(Z)*-coumaroyltaraxerol) isolated from *coula edulis* have anti-hyperglycemic effects, each acting on both reducing starch digestion and stimulating insulin production. This further explains the sugar digestive enzyme inhibitory potential of *coula edulis* extract and provides a good starting point for the development of new multitarget antidiabetic compounds.

Competing Interests: The authors have declared that no competing interests exist.

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