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Integrated Phytochemical, In-Vitro Enzyme Inhibitory, Molecular Docking and ADME Evaluation of Combretum Ovalifolium Roxb. Leaf Extracts as a Potential Source of Antidiabetic Agents

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ABSTRACT:

Background: Diabetes mellitus remains a major metabolic disorder for which identification of complementary and novel therapeutic agents is an active area of research. Medicinal plants contain chemically diverse constituents that may modulate carbohydrate-digesting enzymes and other antidiabetic targets.

Objective: The present study evaluated the phytochemical profile and in vitro carbohydrate-hydrolysing enzyme inhibitory activity of petroleum ether, chloroform and ethanolic leaf extracts of Combretum ovalifolium Roxb., supported by HPTLC fingerprinting, molecular docking against human dipeptidyl peptidase-IV (DPP-IV) and in silico drug-likeness/ADME assessment.

Methods: Leaf extracts were prepared by Soxhlet extraction and subjected to preliminary phytochemical screening. The ethanolic extract was fingerprinted by HPTLC using silica gel 60 F254 plates and toluene:ethyl acetate:formic acid (5:4:1, v/v/v). α-Amylase, α-glucosidase and β-galactosidase inhibition were assessed at 10–500 µg/mL. Twenty phytoconstituents reported from the genus Combretum were docked against DPP-IV (PDB ID: 2ONC) using AutoDock Vina/PyRx. SwissADME was used to assess physicochemical and drug-likeness parameters.

Results: The ethanolic extract showed a broad phytochemical profile and produced three major HPTLC peaks at Rf 0.096, 0.153 and 0.920 at 366 nm. For α-amylase, IC50 values were 61.35, 138.00 and 86.23 µg/mL for petroleum ether, chloroform and ethanol extracts, respectively. For α-glucosidase, the corresponding IC50 values were 139.20, 74.44 and 58.00 µg/mL, while β-galactosidase IC50 values were 70.74, 78.93 and 64.97 µg/mL. In docking, arjunic acid (-15.7 kcal/mol) and betulinic acid (-14.5 kcal/mol) showed the most favourable predicted binding affinities, compared with metformin (-8.6 kcal/mol). Quercetin, luteolin, kaempferol, apigenin, genkwanin and several other compounds complied with Lipinski criteria, whereas larger compounds such as punicalagin showed multiple violations.

Conclusion: C. ovalifolium leaf extracts demonstrated measurable in vitro inhibition of three carbohydrate-related enzymes, while selected Combretum-derived phytoconstituents showed favourable predicted DPP-IV binding and variable drug-likeness. The findings support further chemical identification, target-based validation and in vivo investigation, but do not by themselves establish clinical antidiabetic efficacy.

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by elevated blood glucose resulting from inadequate insulin secretion, impaired insulin action, or both. The global burden continues to increase; the World Health Organization reported that the number of people living with diabetes increased from approximately 200 million in 1990 to 830 million in 2022. The International Diabetes Federation's 2025 estimates similarly indicate that 589 million adults aged 20–79 years were living with diabetes, with further growth projected over the coming decades [1,2]. Persistent hyperglycaemia is associated with vascular and neurological complications, including kidney disease, visual impairment, cardiovascular disease and stroke [1].

Current pharmacological management includes metformin, insulin secretagogues, sodium-glucose cotransporter-2 inhibitors, glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-IV (DPP-IV) inhibitors. Although these agents are effective, treatment remains limited in many settings by cost, access, tolerability, adherence and the need for combination therapy. These limitations continue to stimulate investigation of natural products as sources of structurally diverse molecules with complementary mechanisms of glucose regulation.

Inhibition of intestinal carbohydrate-digesting enzymes is one established strategy for attenuating postprandial glucose excursions. α-Amylase initiates starch hydrolysis by cleaving internal α-1,4 glycosidic bonds, whereas α-glucosidase catalyses the terminal release of glucose from oligosaccharides. In vitro inhibition of these enzymes can therefore provide an initial indication of the ability of a plant extract to slow carbohydrate hydrolysis. β-Galactosidase hydrolyses β-galactosides and has also been investigated as a carbohydrate-processing target in natural-product research [3,4].

The genus Combretum (Combretaceae) is chemically diverse and includes approximately 370 species, with members occurring widely in tropical and subtropical regions. Previous reviews document triterpenes, triterpenoid glycosides, flavonoids, tannins, phenolic compounds and stilbenoids, including combretastatin-type compounds, among the constituents reported from Combretum species [5,6]. Several members of the genus have been investigated for antioxidant, antimicrobial, anti-inflammatory and other pharmacological activities, providing a rationale for further investigation of less-studied species.

Combretum ovalifolium Roxb. is the plant investigated in the present work. The uploaded experimental records show that its leaves yielded chemically distinct petroleum ether, ethyl acetate, chloroform, ethanol and aqueous preparations, with the ethanolic extract giving the highest reported yield among these preparations. The preliminary screening also demonstrated variation in carbohydrate, protein, steroid, glycoside, saponin, alkaloid, tannin and flavonoid reactions among the extracts.

An integrated experimental-computational strategy can strengthen preliminary natural-product investigations. HPTLC provides a reproducible chemical fingerprint for crude extracts, while molecular docking can be used as a hypothesis-generating approach to compare predicted ligand–target interactions. AutoDock Vina provides a commonly used scoring framework for docking and virtual screening [7]. SwissADME provides physicochemical, pharmacokinetic and drug-likeness predictions that can be used to prioritize compounds for subsequent experimental study [8]. Lipinski's Rule of Five remains a useful early filter for oral drug-likeness, although it should not be interpreted as a definitive predictor of efficacy or safety [9].

The present study therefore aimed to characterize C. ovalifolium leaf extracts by preliminary phytochemical screening and HPTLC fingerprinting, determine their in vitro inhibitory activity against α-amylase, α-glucosidase and β-galactosidase, and explore the predicted interaction of twenty Combretum-derived phytoconstituents with human DPP-IV. Drug-likeness and ADME-related properties were subsequently examined to identify compounds that warrant further experimental investigation.

2. MATERIALS AND METHODS

2.1. Plant material, collection and authentication

Fresh leaves of Combretum ovalifolium Roxb. were collected in Tamil Nadu, India, and botanically authenticated at the Xavier Research Foundation (XRF), St Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India. The authentication certificate, dated September 2024, identifies the plant as Combretum ovalifolium Roxb. (Family: Combretaceae) and was issued by Dr. S. Mutheswaran, Scientist, Xavier Research Foundation. The authenticated plant material was washed with distilled water, air-dried/shade-dried and powdered before extraction.

2.2. Physicochemical evaluation and preparation of extracts

The uploaded protocol records physicochemical evaluation of the powdered leaf material, including total ash, water-soluble ash, acid-insoluble ash, sulphated ash, loss on drying, water-soluble extractive and alcohol-soluble extractive values. The reported values were 3.73%, 6.70%, 1.93%, 7.20%, 8.97%, 16.34% and 14.02%, respectively.

According to the extraction protocol, approximately 200 g of dried powdered leaves were subjected to continuous Soxhlet extraction with petroleum ether, chloroform, ethyl acetate and ethanol (99.9%) for 18 h. The aqueous preparation was obtained by decoction. Extracts were filtered while hot, concentrated using a rotary vacuum evaporator and dried before storage in a desiccator. Reported yields were 4.09% for petroleum ether, 5.68% for ethyl acetate, 4.69% for chloroform, 6.64% for ethanol and 4.90% for the aqueous preparation. The enzyme inhibition experiments were performed on petroleum ether, chloroform and ethanol extracts.

2.3. Preliminary phytochemical screening

Qualitative phytochemical screening was performed using standard pharmacognostic tests. Carbohydrates were assessed by Molisch's, Fehling's and Barfoed's tests; proteins by Biuret and Millon's tests; amino acids by the ninhydrin test; steroids by Liebermann–Burchard and Salkowski tests; glycosides by Keller–Killiani and Borntrager's tests; saponins by the foam test; alkaloids by Mayer's, Hager's, Wagner's and Dragendorff's tests; tannins by lead acetate, ferric chloride and acetic acid tests; and flavonoids by Shinoda and sulphuric acid tests. Results were recorded as positive or negative reactions.

2.4. HPTLC fingerprinting

HPTLC analysis of the ethanolic extract was performed using a CAMAG Linomat 5 sample applicator and TLC Scanner 4. The extract was prepared at 1 mg/mL in chromatographic-grade ethanol, filtered, sonicated and centrifuged. Aliquots of 5, 10, 15 and 20 µL were applied as bands on Merck silica gel 60 F254 aluminium plates (100 × 100 mm). Chromatographic development was performed in a twin-trough chamber saturated for 20 min with toluene:ethyl acetate:formic acid (5:4:1, v/v/v), to a solvent-front distance of 70 mm. The plates were air-dried and scanned in absorbance mode at 254 and 366 nm. The source HPTLC report documented three principal peaks at Rf 0.096, 0.153 and 0.920 at 366 nm [10].

2.5. α-Amylase inhibition assay

α-Amylase inhibition was evaluated using the DNS method with modifications of the protocol reported in the assay records. Extract concentrations of 10, 50, 100, 250 and 500 µg/mL were pre-incubated with 200 µL α-amylase (1.0 U/mL in phosphate buffer, pH 6.9) at 25 °C for 30 min. Then 400 µL of 0.25% starch solution was added and the reaction was incubated at 37 °C for 5 min. The reaction was terminated with 1.0 mL DNS reagent containing 1% 3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH. Tubes were heated in a boiling water bath for 10 min, cooled and diluted to 10 mL with distilled water. Absorbance was measured at 540 nm. Acarbose was used as the reference standard. Percentage inhibition was calculated using the blank-corrected equation: % inhibition = [Acontrol − (Atest − Ablank)]/Acontrol × 100 [11].

2.6. α-Glucosidase inhibition assay

α-Glucosidase inhibition was assessed using p-nitrophenyl-α-D-glucopyranoside (PNPG) as substrate. Extract concentrations of 10–500 µg/mL were incubated with α-glucosidase (0.2 U/mL) in 0.1 M phosphate buffer (pH 6.8) at 37 °C for 30 min. PNPG (5 mM) was then added and the reaction was incubated for a further 20 min. The reaction was stopped using 160 µL of 0.2 M sodium carbonate, and absorbance was measured at 405 nm. Acarbose was used as positive control. Percentage inhibition was calculated as [(Acontrol − Asample)/Acontrol] × 100. The assay records identify the method as based on Ahamad et al.

(2010); the complete bibliographic details of that reference should be verified against the laboratory's original method source before submission.

2.7. β-Galactosidase inhibition assay

β-Galactosidase inhibition was evaluated using o-nitrophenyl-β-D-galactopyranoside (ONPG). Extract concentrations of 10–500 µg/mL were pre-incubated with β-galactosidase in sodium acetate buffer (pH 5.5) at room temperature for 20 min. A substrate mixture containing 8.3 mM ONPG, 1 mM MgCl2 and 0.1 M β-mercaptoethanol in 0.1 M sodium phosphate buffer (pH 7.0) was added, followed by incubation at 30 °C for 20 min. The reaction was stopped with 0.5 M sodium carbonate, and o-nitrophenol formation was measured at 420 nm using a microplate reader. Metformin was used as the reference drug. Each concentration was assessed in three biological replicates.

2.8. Molecular docking

Twenty phytoconstituents reported from the genus Combretum were selected by literature survey; they were not analytically identified in the present C. ovalifolium extract. Metformin was included as the reference compound. Three-dimensional ligand structures were obtained from PubChem, converted to PDB format using BIOVIA Discovery Studio Visualizer, energy-minimized using Open Babel and saved as PDBQT files.

The crystal structure of human DPP-IV (PDB ID: 2ONC) was obtained from the Protein Data Bank. Water molecules, cofactors and other heteroatoms were removed before docking. Polar hydrogens and Kollman charges were added, and the prepared receptor was saved in PDBQT format. Docking was performed using AutoDock Vina through the PyRx virtual-screening platform. Binding affinity values were reported in kcal/mol, with more negative scores interpreted as more favourable predicted binding. Protein–ligand interactions were visualized using BIOVIA Discovery Studio Visualizer [7]. The uploaded docking report does not document a redocking/RMSD validation procedure; therefore, the docking results are interpreted as virtual-screening predictions rather than experimentally validated binding modes.

2.9. Drug-likeness and ADME prediction

SwissADME was used to evaluate molecular weight, lipophilicity (LogP), topological polar surface area (TPSA), hydrogen-bond acceptors (HBA), hydrogen-bond donors (HBD), and Lipinski rule violations. Additional pharmacokinetic outputs in the ADMET source file included gastrointestinal absorption, blood–brain barrier permeability, P-glycoprotein substrate status, selected cytochrome P450 inhibition predictions, skin permeation, bioavailability score and medicinal-chemistry alerts. SwissADME is a computational prioritization tool and its predictions were not treated as experimental evidence of safety or efficacy [8].

2.10. Statistical analysis

All experimental measurements were performed in triplicate and expressed as mean ± standard deviation (SD). The inhibitory activities of the petroleum ether, chloroform and ethanolic extracts at different concentrations were analyzed statistically using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparison test for post-hoc comparisons. A probability value of $p < 0.05$ was considered statistically significant. IC_{50} values were determined by nonlinear dose–response regression analysis using GraphPad Prism.

3. RESULTS

3.1. Physicochemical characteristics, extract yield and preliminary phytochemical screening

The powdered leaves showed total ash of 3.73%, water-soluble ash of 6.70%, acid-insoluble ash of 1.93%, sulphated ash of 7.20%, loss on drying of 8.97%, water-soluble extractive of 16.34% and alcohol-soluble extractive of 14.02%. Among the prepared extracts, the ethanolic extract showed the highest reported yield (6.64%), followed by ethyl acetate (5.68%), aqueous (4.90%), chloroform (4.69%) and petroleum ether (4.09%) (Table 1).

Table 1. Extractive yield of C. ovalifolium leaf preparations.

Extract	Appearance	Yield (% w/w)
Petroleum ether	Green, viscous	4.09

Ethyl acetate	Green, viscous	5.68
Chloroform	Green, sticky	4.69
Ethanol	Green, semisolid	6.64
Aqueous	Brown, viscous	4.90

Preliminary screening demonstrated marked solvent-dependent variation. The ethanolic extract was positive for carbohydrates, proteins, steroids, alkaloid tests (Wagner and Dragendorff), tannins and flavonoids. The petroleum ether extract showed steroidal, glycosidic, saponin and carbohydrate reactions, whereas the ethyl acetate extract showed a broad profile including carbohydrates, proteins, glycosides, alkaloids and tannins. The aqueous extract showed carbohydrates, proteins, alkaloids and flavonoids/tannins by the recorded tests. Amino acids were negative in all tested extracts (Table 2).

Table 2. Preliminary phytochemical screening of C. ovalifolium extracts. +, positive reaction; –, negative reaction.

Phytochemical test	**Petroleum ether**	**Ethyl acetate**	**Chloroform**	**Ethanol**	**Aqueous**
Molisch carbohydrate	+	+	–	+	+
Fehling carbohydrate	–	+	–	+	+
Barfoed carbohydrate	+	+	–	+	–
Biuret protein	–	+	+	+	+
Millon's protein	–	+	+	+	+
Ninhydrin amino acids	–	–	–	–	–
Liebermann–Burchard steroids	+	–	–	+	–
Salkowski steroids	+	–	–	+	–
Keller–Killiani glycosides	–	+	–	–	–
Borntrager's glycosides	+	+	–	–	–
Foam saponins	+	–	+	–	–
Mayer alkaloids	–	+	–	–	+
Hager alkaloids	–	–	–	–	–
Wagner alkaloids	–	+	+	+	+
Dragendorff alkaloids	–	+	+	+	+
Lead acetate tannins	–	+	–	+	–

Ferric chloride tannins	–	+	–	+	+
Acetic acid tannins	–	+	–	–	–
Shinoda flavonoids	–	–	–	+	+
Sulphuric acid flavonoids	–	–	–	–	–

3.2. HPTLC fingerprint analysis

The ethanolic extract produced a characteristic HPTLC fingerprint on silica gel 60 F254 plates using toluene:ethyl acetate:formic acid (5:4:1, v/v/v). The source chromatographic report documented three principal peaks at 366 nm with Rf values of 0.096, 0.153 and 0.920. The chromatographic record also documents CAMAG Linomat 5 application, TLC Scanner 4 detection, 20-min chamber saturation and a 70-mm solvent-front distance. The three peaks should be regarded as chromatographic markers rather than identified compounds because no co-chromatography with authentic standards or mass-spectrometric identification was reported (Table 3) (Figure 1-3).

Peak	**Wavelength**	**Rf value**	**Interpretation**
1	366 nm	0.096	Major chromatographic peak
2	366 nm	0.153	Major chromatographic peak
3	366 nm	0.920	Major chromatographic peak

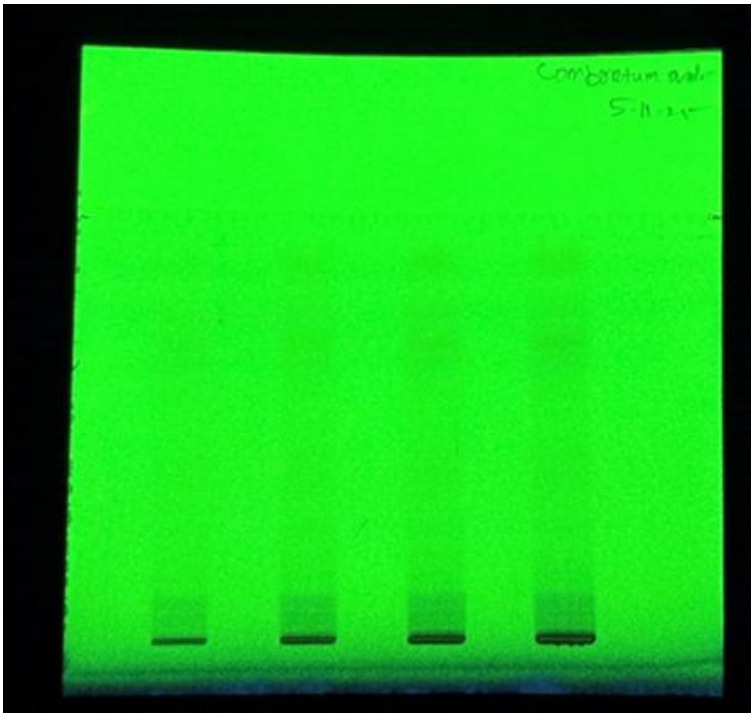

Figure 1. HPTLC chromatogram of C. ovalifolium ethanolic extract at 254 nm.

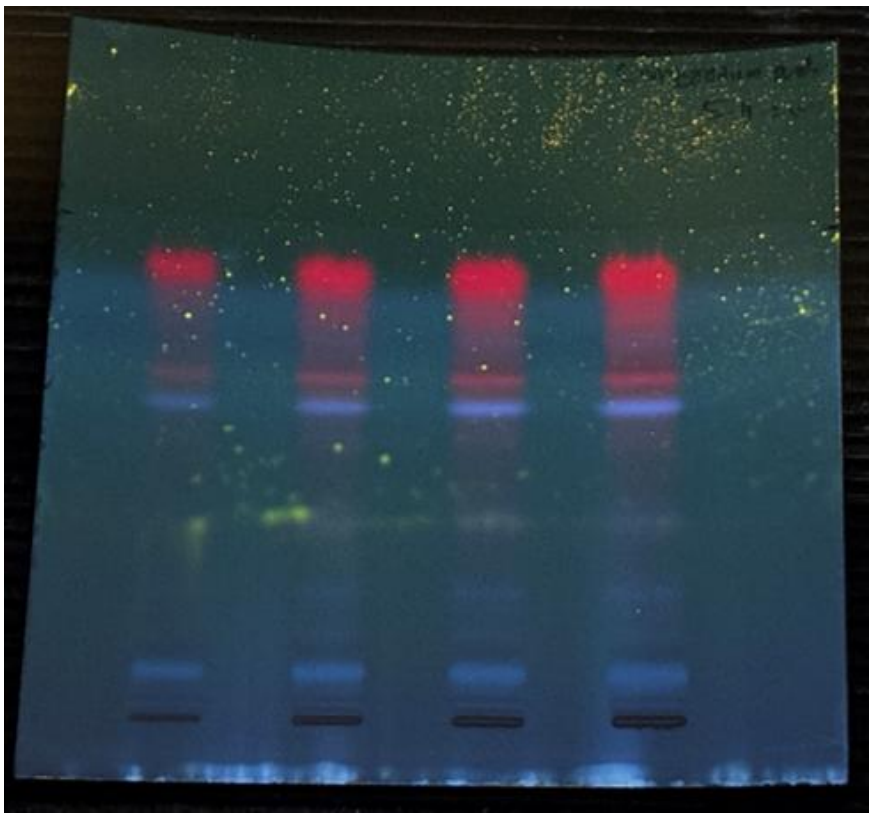

Figure 2. HPTLC chromatogram of C. ovalifolium ethanolic extract at 366 nm.

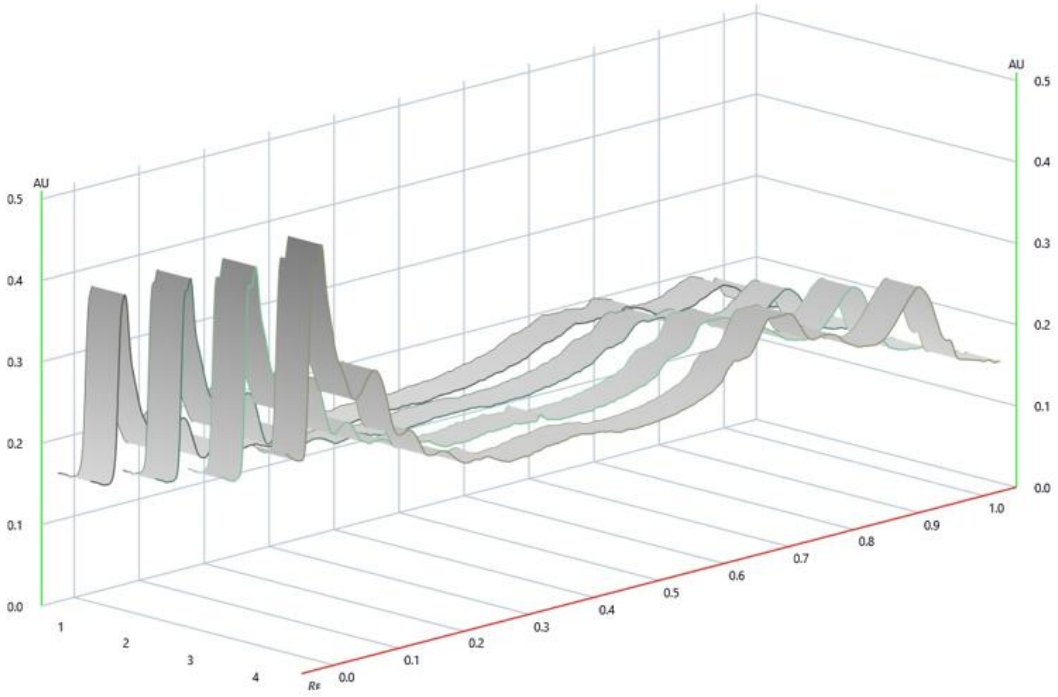

Figure 3. HPTLC densitogram of C. ovalifolium ethanolic extract.

3.3. α-Amylase inhibitory activity

All three tested extracts inhibited α-amylase in the concentration range of 10–500 µg/mL. The petroleum ether extract was the most potent based on IC50 (61.35 µg/mL), followed by ethanol (86.23 µg/mL) and chloroform (138.00 µg/mL). At 500 µg/mL, inhibition reached 71.36% for petroleum ether, 66.88% for chloroform and 66.99% for ethanol, compared with 74.89% for the reference standard. The nonlinear regression fits reported in the source files gave R^2 values of 0.9714, 0.8943 and 0.8703 for petroleum ether, chloroform and ethanol, respectively (Table 4 & 5) (Figure 4) .

Table 4. α-Amylase inhibition (%) by C. ovalifolium extracts. Values are mean ± SD (n = 3).

Concentration (µg/mL)	Petroleum ether	Chloroform	Ethanol
500	71.36 ± 1.35	66.88 ± 0.91	66.99 ± 0.61
250	68.70 ± 0.54	62.97 ± 1.93	65.42 ± 0.18
100	59.62 ± 1.36	60.90 ± 0.90	64.89 ± 0.28
50	55.61 ± 0.48	58.77 ± 0.70	63.96 ± 0.32
10	40.93 ± 1.75	56.85 ± 0.44	62.15 ± 0.87
Standard	74.89 ± 2.12	67.38 ± 0.92	67.38 ± 0.92

Table 5. IC50 values for α-amylase inhibition.

Extract	IC50 (µg/mL)	R^2
Petroleum ether	61.35	0.9714
Chloroform	138.00	0.8943
Ethanol	86.23	0.8703

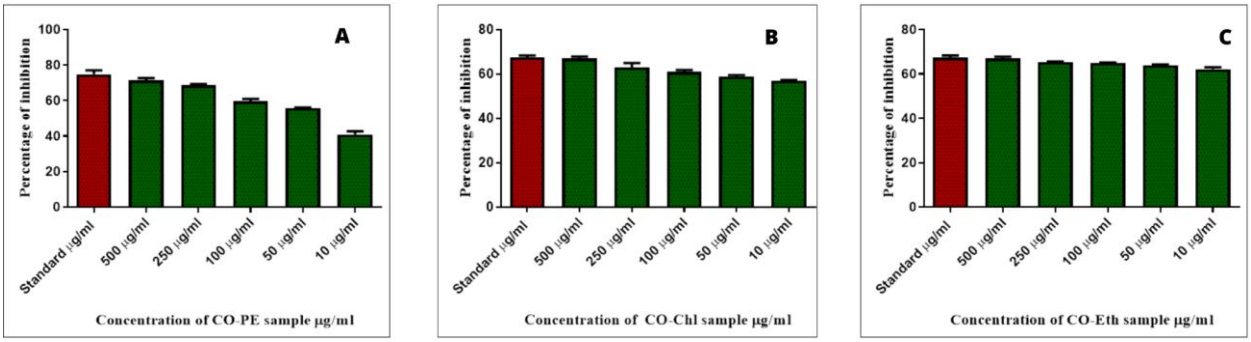

Figure 4. Concentration-dependent α-amylase inhibitory activity of C. ovalifolium leaf extracts. (A) Petroleum ether, (B) chloroform and (C) ethanolic extract. Bars represent mean ± SD (n = 3).

3.4. α-Glucosidase inhibitory activity

The three extracts also inhibited α-glucosidase in a concentration-dependent manner. The ethanolic extract showed the lowest IC50 (58.00 µg/mL), followed by chloroform (74.44 µg/mL) and petroleum ether (139.20 µg/mL). At 500 µg/mL, the recorded inhibition was 86.33% for petroleum ether, 86.29% for chloroform and 85.45% for ethanol. Thus, the ranking at the highest tested concentration was not identical to the IC50 ranking, emphasizing the importance of comparing the complete dose–response relationship rather than a single concentration (Table 6 & 7) (Figure 5).

Table 6. α-Glucosidase inhibition (%) by C. ovalifolium extracts. Values are mean ± SD (n = 3).

Concentration (µg/mL)	Petroleum ether (%)	Chloroform (%)	Ethanol (%)
500	86.33 ± 1.21	86.29 ± 0.05	85.45 ± 0.60
250	79.28 ± 1.52	83.88 ± 0.56	84.23 ± 3.25
100	75.02 ± 1.90	80.32 ± 0.64	83.14 ± 0.40
50	70.83 ± 0.53	77.32 ± 1.36	82.23 ± 2.91
10	67.57 ± 2.14	71.70 ± 1.62	78.79 ± 4.82
Standard	91.05 ± 0.58	91.00 ± 0.58	81.60 ± 0.99

Table 7. IC50 values for α-glucosidase inhibition.

Extract	IC50 (µg/mL)	R^2
Petroleum ether	139.20	0.9155
Chloroform	74.44	0.9640
Ethanol	58.00	0.7586

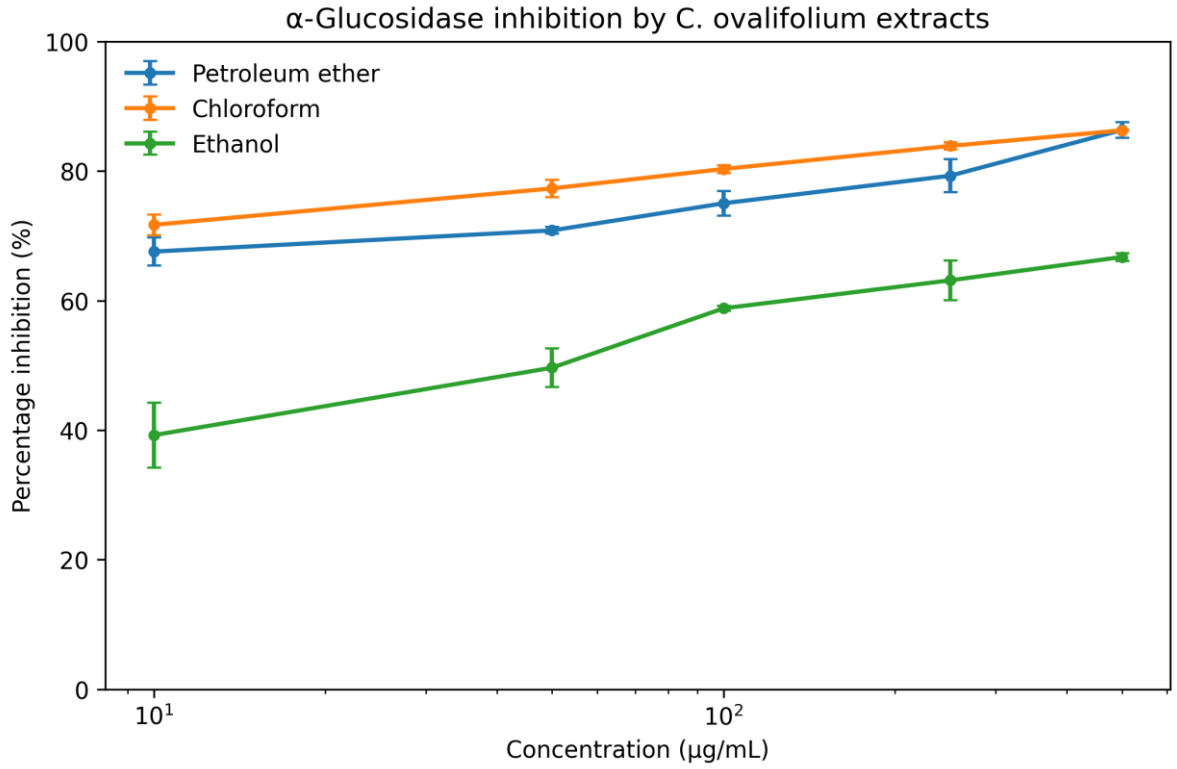

Figure 5. Concentration-dependent α-glucosidase inhibition by C. ovalifolium extracts. Data are mean ± SD (n = 3); concentration is shown on a logarithmic x-axis.

3.5. β-Galactosidase inhibitory activity

β-Galactosidase activity was inhibited by all three extracts across the tested concentration range. The lowest IC50 was observed for the ethanolic extract (64.97 µg/mL), followed by petroleum ether (70.74 µg/mL) and chloroform (78.93 µg/mL). At 500 µg/mL, petroleum ether produced the highest recorded inhibition (77.38%), followed by chloroform (70.47%) and ethanol (66.73%). The IC50-based ranking and single-concentration ranking therefore differed, again highlighting the importance of the dose–response analysis (Table 8 & 9) (Figure 6).

Table 8. β-Galactosidase inhibition (%) by C. ovalifolium extracts. Values are mean ± SD (n = 3).

Concentration (µg/mL)	Petroleum ether (%)	Chloroform (%)	Ethanol (%)
500	77.38 ± 1.03	70.47 ± 1.15	66.73 ± 0.60
250	72.90 ± 2.30	65.57 ± 2.77	63.13 ± 3.25
100	68.67 ± 2.78	60.84 ± 0.98	58.81 ± 0.40
50	63.60 ± 4.11	58.82 ± 0.65	49.64 ± 2.91
10	54.30 ± 2.66	50.00 ± 5.61	39.23 ± 4.82
Standard	84.31 ± 2.08	84.83 ± 1.85	81.60 ± 0.99

Table 9. IC50 values for β-galactosidase inhibition.

Extract	IC50 (µg/mL)	R^2
Petroleum ether	70.74	0.9398
Chloroform	78.93	0.8551
Ethanol	64.97	0.9370

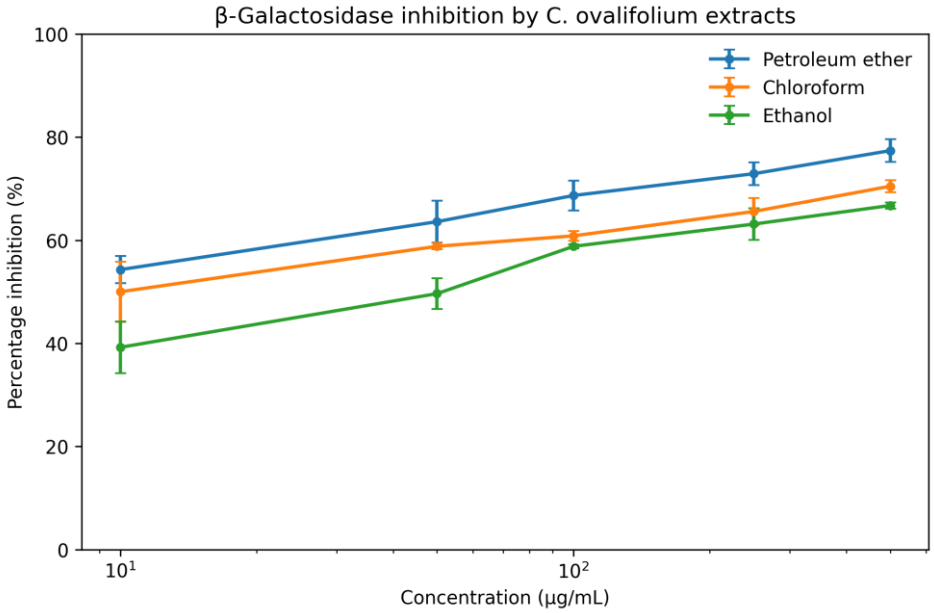

Figure 6. Concentration-dependent β-galactosidase inhibition by C. ovalifolium extracts. Data are mean ± SD (n = 3); concentration is shown on a logarithmic x-axis.

3.6. Molecular docking analysis

Docking of twenty Combretum-derived phytoconstituents against human DPP-IV (PDB ID: 2ONC) produced predicted binding affinities from −7.8 to −15.7 kcal/mol. Arjunic acid showed the most negative score (−15.7 kcal/mol), followed by betulinic acid (−14.5 kcal/mol). Punicalagin and combretastatin A4 also showed favourable predicted scores of −10.9 and −10.0 kcal/mol, respectively. Metformin showed a predicted affinity of −8.6 kcal/mol in the same docking workflow (Table 10) (Figure 7-12).

Table 10. Predicted binding affinities of Combretum-derived phytoconstituents and metformin against DPP-IV (PDB ID: 2ONC).

Compound	**Binding affinity (kcal/mol)**
Arjunic acid	−15.7
Betulinic acid	−14.5
Punicalagin	−10.9
Combretastatin A4	−10.0
α-Amyrin	−9.6
Cauloside	−9.2
Arjunolic acid	−9.1
Lupeol	−9.0
Myricetin	−8.7
Isovitexin	−8.6
Arjungenin	−8.6
Luteolin	−8.4
Vitexin	−8.4
Quercetin	−8.3
Rhamnetin	−8.3
Rhamnocitrin	−8.3
Genkwanin	−8.2
Kaempferol	−8.1
Apigenin	−7.8
Standard (Metformin)	−8.6

The strongest docking scores were observed for triterpenoid-type compounds, particularly arjunic acid and betulinic acid. These values indicate favourable computational scoring within the selected docking protocol, but they should not be interpreted as direct measures of inhibitory potency or as evidence that the compounds are present in the tested C. ovalifolium extract.

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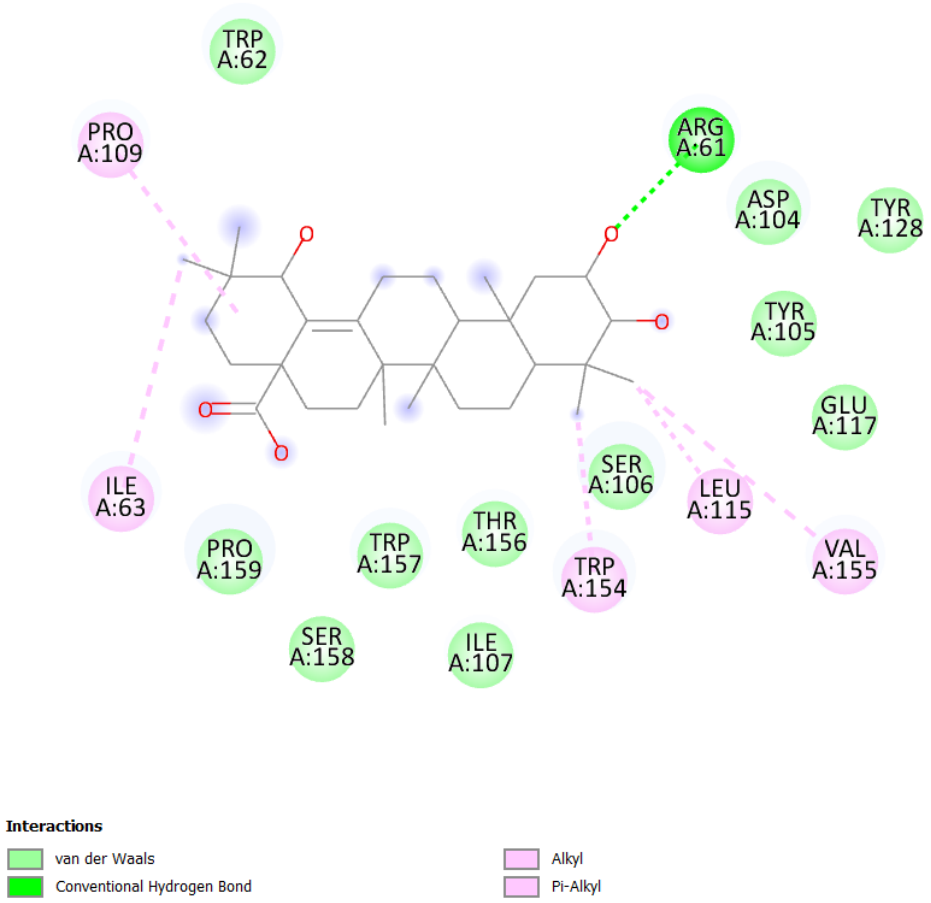

Figure 7. Two-dimensional interaction profile of arjunic acid with DPP-IV (PDB ID: 2ONC).

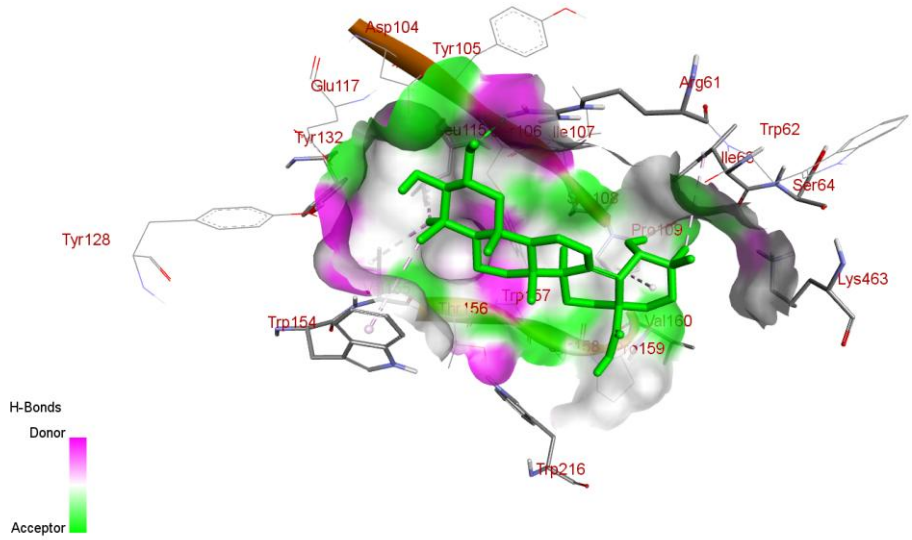

Figure 8. Three-dimensional interaction profile of arjunic acid with DPP-IV (PDB ID: 2ONC).

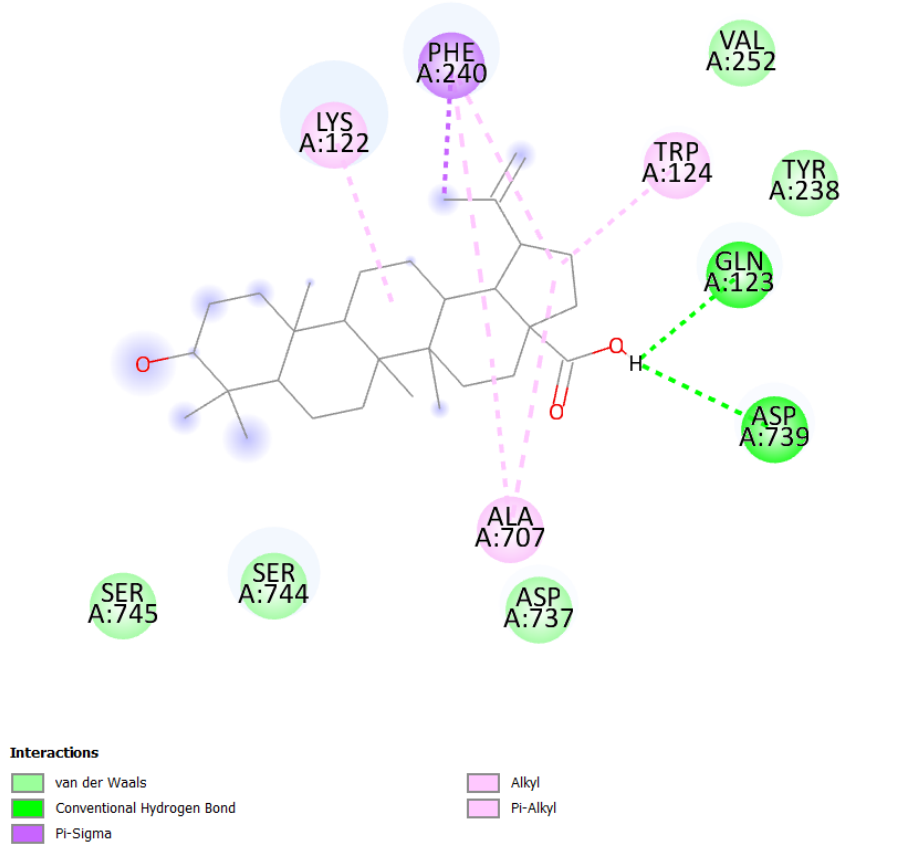

Figure 9. Two-dimensional interaction profile of betulinic acid with DPP-IV (PDB ID: 2ONC).

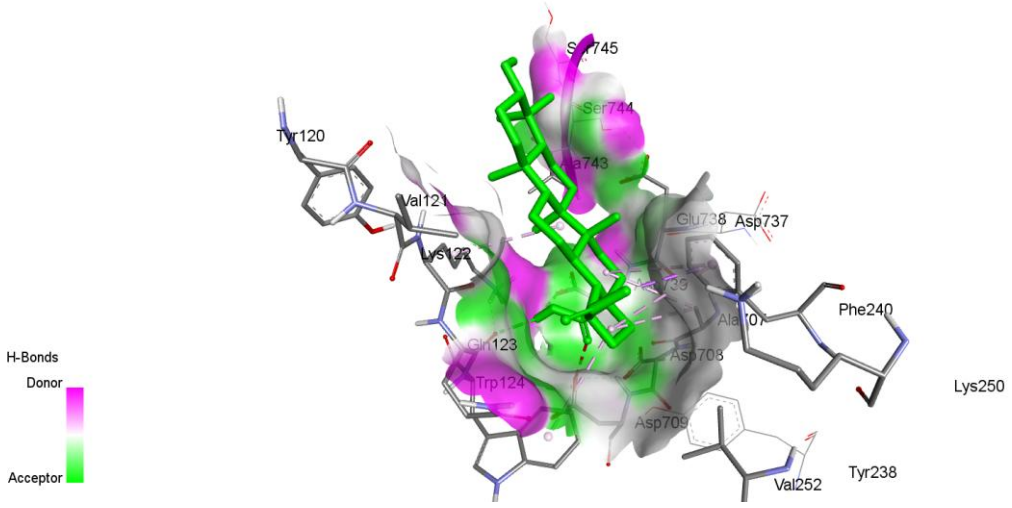

Figure 10. Three-dimensional interaction profile of betulinic acid with DPP-IV (PDB ID: 2ONC).

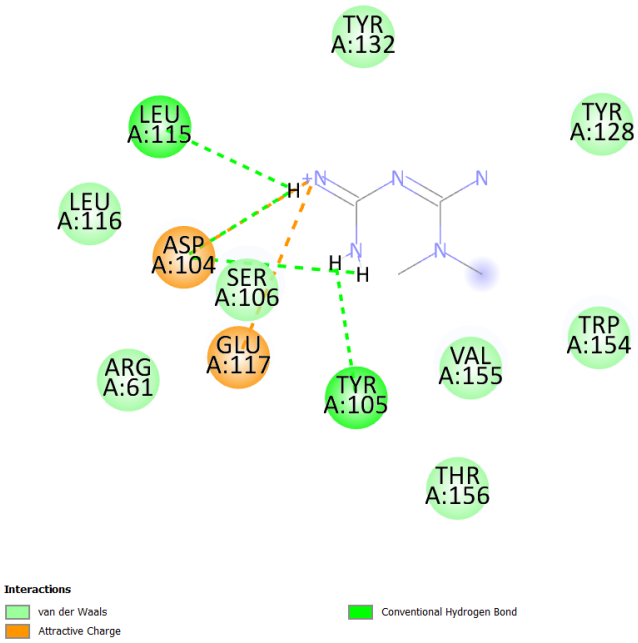

Figure 11. Two-dimensional interaction profile of Metformin (standard drug) with human DPP-IV (PDB ID: 2ONC).

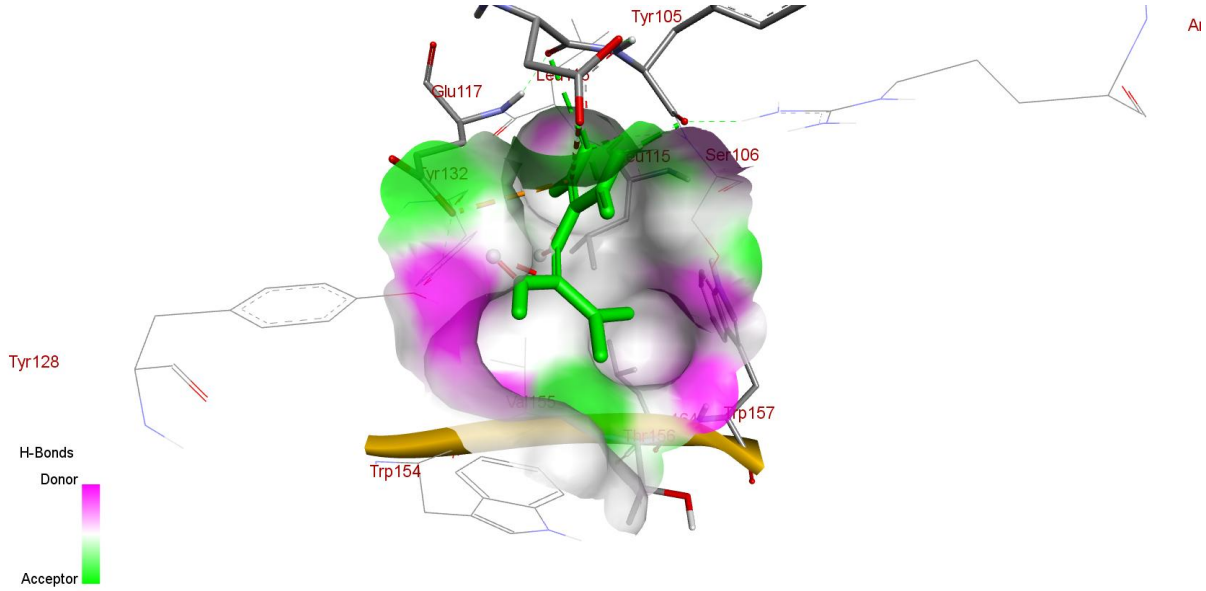

Figure 12. Three-dimensional interaction profile of Metformin (standard drug) with human DPP-IV (PDB ID: 2ONC).

3.7. Drug-likeness and ADME prediction

The SwissADME analysis showed substantial variation in physicochemical properties. Quercetin, luteolin, kaempferol, apigenin, genkwanin, rhamnetin, rhamnocitrin, combretastatin A4 and arjunic acid had no reported Lipinski violations in the docking report. Betulinic acid, α-amyrin and arjunolic acid also complied with the reported Lipinski criteria. Conversely, lupeol, cauloside, vitexin, myricetin, 6β-hydroxyarjunic acid and arjungenin showed one violation, whereas punicalagin showed three violations (Table 11).

Table 11. Physicochemical and drug-likeness parameters reported by the SwissADME/docking report.

Compound	LogP	TPSA (Å²)	MW (g/mol)	HBA	HBD	Lipinski violations
Betulinic acid	3.83	57.53	456.70	3	2	0
α-Amyrin	4.80	20.23	426.72	1	1	0
Arjunolic acid	3.11	97.99	488.70	5	4	0
Isovitexin	1.60	181.05	432.38	10	7	0
Lupeol	4.72	20.23	426.72	1	1	1
Cauloside	3.83	136.68	604.81	8	5	1
Quercetin	1.63	131.36	302.24	7	5	0
Vitexin	1.63	181.05	432.38	10	7	1
Luteolin	1.86	111.13	286.24	6	4	0
Kaempferol	1.70	111.13	286.24	6	4	0
Apigenin	1.89	90.90	270.24	5	3	0
Genkwanin	2.48	79.90	284.26	5	2	0
Myricetin	1.08	151.59	318.24	8	6	1
Rhamnetin	2.23	120.36	316.26	7	4	0
Rhamnocitrin	2.31	100.13	300.26	6	3	0
Combretastatin A4	3.30	77.38	334.36	6	2	0
6β-Hydroxyarjunic acid	2.89	118.22	504.70	6	5	1
Arjungenin	3.08	118.22	504.70	6	5	1
Arjunic acid	3.31	97.99	488.70	5	4	0
Punicalagin	0.42	526.60	1084.72	30	17	3
Metformin	0.34	91.49	129.16	2	3	0

Punicalagin illustrates the distinction between docking score and drug-likeness: despite a favourable predicted binding affinity (−10.9 kcal/mol), its molecular weight (1084.72 Da), TPSA (526.60 Å²), 30 hydrogen-bond acceptors and 17 hydrogen-bond donors produced three Lipinski violations. Such properties are generally unfavourable for passive oral absorption. In contrast, smaller flavonoids such as apigenin, genkwanin, kaempferol, luteolin and quercetin showed no reported Lipinski violations, although high polarity and hydrogen-bonding capacity may still influence permeability and bioavailability.

The detailed ADMET source file also shows that individual compounds can differ markedly in predicted gastrointestinal absorption, BBB permeability, P-glycoprotein substrate status and cytochrome P450 interactions. These predictions should be regarded as prioritization data rather than evidence of clinical pharmacokinetics or toxicity.

4. DISCUSSION

The present investigation provides an integrated preliminary assessment of C. ovalifolium leaves using phytochemical screening, HPTLC fingerprinting, three enzyme inhibition assays and computational target exploration. The study is strengthened by the use of multiple solvent extracts and concentration-dependent enzyme testing, while the main limitations are the absence of direct compound identification in the extract, absence of in vivo validation, and lack of experimentally validated DPP-IV inhibition.

The extraction results demonstrate a clear influence of solvent polarity. Ethanol produced the highest yield among the five preparations (6.64%), consistent with its ability to recover a broad range of polar and moderately polar constituents. The phytochemical screen supports this interpretation, with the ethanolic extract showing positive reactions for several major classes, including flavonoids, tannins, alkaloid tests, proteins and carbohydrates. Combretum species are known to contain diverse triterpenoid and phenolic constituents, including flavonoids and tannins, which provides a plausible chemical context for the observed biological activity [5,6]. However, qualitative phytochemical tests cannot establish concentrations or identify individual compounds, and the observed reactions should therefore be considered preliminary.

The HPTLC fingerprint provides a useful quality-control layer for the ethanolic extract. Three reproducible principal peaks were documented at Rf 0.096, 0.153 and 0.920 at 366 nm. Because no authentic standards were co-chromatographed, these peaks should not be assigned to specific compounds. Nevertheless, the fingerprint can be useful for batch comparison and future standardization. A subsequent LC-MS/MS or HPLC-DAD study would be required to connect individual chromatographic peaks with molecular identities.

All three extracts inhibited α-amylase, with petroleum ether giving the lowest IC50 (61.35 µg/mL). The same extract reached 71.36% inhibition at 500 µg/mL, close to the recorded standard response of 74.89%. The DNS-based assay is widely used for preliminary screening of natural α-amylase inhibitors because it measures reducing sugars generated from starch hydrolysis [11]. The stronger activity of the petroleum ether extract suggests that relatively lipophilic constituents may contribute to α-amylase inhibition. The ethanolic extract also showed substantial activity (IC50 86.23 µg/mL), consistent with its broader phytochemical profile.

For α-glucosidase, the ethanolic extract had the lowest IC50 (58.00 µg/mL), followed by chloroform (78.93 µg/mL) and petroleum ether (139.20 µg/mL). This difference from the α-amylase ranking is biologically relevant because α-amylase and α-glucosidase have distinct catalytic roles and can respond differently to chemically diverse inhibitors. Similar natural-product studies have reported concentration-dependent α-glucosidase inhibition and used PNPG-based spectrophotometric assays at 405 nm [12,13]. The current results therefore suggest that the ethanolic extract may contain constituents with greater relative affinity for α-glucosidase than for α-amylase.

β-Galactosidase was also inhibited, with IC50 values of 64.97, 70.74 and 78.93 µg/mL for ethanol, petroleum ether and chloroform, respectively. ONPG-based β-galactosidase inhibition assays are established in natural-product studies [3]. However, β-galactosidase should be presented as a complementary carbohydrate-hydrolysing target rather than equated directly with the principal intestinal α-glucosidase pathway. The current assay demonstrates enzyme inhibition under the experimental conditions, but additional mechanistic and physiological studies would be needed to establish its contribution to glucose lowering in vivo.

The docking analysis adds a target-based hypothesis for DPP-IV. Arjunic acid and betulinic acid produced the most negative predicted binding scores, −15.7 and −14.5 kcal/mol, respectively, whereas metformin scored −8.6 kcal/mol in the same docking workflow. Combretum species are reported to contain triterpenoids and related compounds, making these scaffold classes chemically plausible members of the broader genus [5,6]. Nevertheless, the docked ligands were selected from compounds reported for the genus rather than demonstrated to be present in the current C. ovalifolium extract. Therefore, the docking results should not be used to claim that arjunic acid or betulinic acid caused the observed enzyme inhibition.

A limitation of the molecular docking analysis is that complete validation parameters, including native-ligand redocking and root-mean-square deviation (RMSD) assessment, were not available in the computational records. Therefore, the docking results should be interpreted as preliminary computational predictions that provide supportive evidence for potential interactions between the selected *Combretum*-derived phytoconstituents and the DPP-IV target. The favourable binding affinities observed for arjunic acid (−15.7 kcal/mol) and betulinic acid (−14.5 kcal/mol) suggest that these compounds may have a

potential to interact strongly with the DPP-IV binding site under the applied docking conditions. However, docking scores alone cannot establish enzyme inhibition or therapeutic efficacy. Further studies involving validated docking protocols, molecular dynamics simulations, direct DPP-IV enzyme inhibition assays, and experimental determination of ligand–protein interactions are required to confirm these computational predictions.

The SwissADME analysis demonstrates why docking score alone is insufficient for lead prioritization. Punicalagin showed a strong predicted docking score but also a very large molecular size and three Lipinski violations. In contrast, several flavonoids, including apigenin, genkwanin, kaempferol, luteolin and quercetin, showed no reported Lipinski violations. The drug-likeness results are nevertheless only preliminary because SwissADME predictions do not establish experimental absorption, metabolism, toxicity or therapeutic efficacy [8,9].

Taken together, the experimental findings support C. ovalifolium as a source of extract-level carbohydrate-enzyme inhibitory activity, with different extracts displaying different apparent potency profiles. The results also generate hypotheses about possible DPP-IV-active phytochemical scaffolds. The next stage should focus on chemical identification of active fractions, activity-guided isolation, direct testing of isolated compounds against α-amylase, α-glucosidase and DPP-IV, followed by in vivo glycaemic evaluation and toxicological assessment.

5. CONCLUSION

C. ovalifolium leaf extracts demonstrated concentration-dependent inhibition of α-amylase, α-glucosidase and β-galactosidase in vitro. Petroleum ether showed the lowest α-amylase IC50, whereas ethanol showed the lowest α-glucosidase and β-galactosidase IC50 values. HPTLC established a characteristic ethanolic-extract fingerprint with three principal peaks at Rf 0.096, 0.153 and 0.920. Among twenty Combretum-derived compounds screened computationally against DPP-IV, arjunic acid and betulinic acid produced the most favourable predicted binding scores. Several smaller flavonoids complied with Lipinski criteria, whereas punicalagin showed substantial drug-likeness limitations. Overall, the study supports further investigation of C. ovalifolium as a source of antidiabetic lead compounds, while emphasizing that extract-level enzyme inhibition and docking predictions require chemical, pharmacological and in vivo validation before therapeutic claims can be made.

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AUTHOR CONTRIBUTIONS

JA Conceptualization, methodology, investigation, writing original draft preparation, VN and NR supervision, review and editing, all authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

This article was based on the phytochemical studies, instrumental analysis, *in – vitro* and *in – silico* studies only, the research work did not involve experimental animals or human participants, and so ethical committee certificate was not required.

DATA AVAILABILITY STATEMENT

All the data produced in this study are available in Supplementary Material.

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