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In-silico discovery of antidiabetic drug potential of *Balanites aegyptiaca* leaf's phenolic compounds

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ABSTRACT: *Balanites aegyptiaca* leaf is very effective in managing diabetes and rich in phenolic compounds. However, the modes of action of the phytochemicals are mainly unknown. Thus, the present in silico drug discovery study on some phenolic compounds was designed to evaluate potential mechanisms of action of the antihyperglycemic phytochemicals of *B. aegyptiaca* leaf extract. The study deployed in silico drug-like studying techniques such as; predicted activity spectra of substances (PASS), molecular docking, prediction of adsorption, distribution, metabolism, excretion, and toxicity (ADMET), Lipinski's rule of 5 (PLOP). The study reveals six compounds with good drug-like properties: cLogp, hydrogen bond donor/acceptor (<5/ <10), and molar refractivity. In addition, ADMET and drug properties like kinase inhibitors, ion channel modulators, and nuclear receptors were positive for the compounds. Each phenolic compound showed one or more antidiabetic activities like insulin promoter, insulin sensitizer and inhibitors of α -amylase and α -glucosidase. Docking result predicted that the phenolic compounds inhibited either α -amylase or α -glucosidase while one of the compounds; 2-methoxy-4-(1-propenyl)-phenol inhibited both α -amylase and α -glucosidase with binding energies of -4.4 and -4.2 kcal/mol against -3.8 and -4.8 kcal/mol by Acarbose. The study revealed that phenolic compounds from *B. aegyptiaca* leaf possessed drug-like properties, including the ability to interact with α -amylase and α -glucosidase, a vital target protein in the management of diabetes mellitus. The data from the in silico study is a step toward the pharmaceutical discovery of the antidiabetic drug potential of *B. aegyptiaca* leaf.

1. INTRODUCTION

Diabetes mellitus is one of the diseases affecting millions of people globally, with a high-frequency rate estimated to reach 592 million people in 2035 (Guariguata et al., 2014). It is a metabolic disorder characterized by severe hyperglycemia resulting from insulin deficiency or insensitivity to target organs. Diabetes mellitus is associated with the promotion of several diseases such as cardiovascular diseases, hypertension etc. which increases the severity of complications to the patients (Mirza et al., 2013). Treatment of diabetes mellitus with available drugs is still not satisfactory and associated with adverse side effects (Ganesan et al., 2021). The challenges associated with the user of the current drugs has prompted the demand and dependence on alternative sources like natural products which have few or no side effect and high efficacy.

The plant '*Balanites aegyptiaca*' is one of the medicinal plants used by traditional medicine practitioners in Nigeria to manage diabetes mellitus (Abubakar et al., 2017). Several

studies have shown that extract from various parts of the plant is a valuable source of hypoglycemic remedy. They were found to promote insulin secretion, inhibit amylase and glucosidase, increase muscle basal glucose uptake, and regulate glucose metabolic enzymes (Gad et al., 2006; Mhya et al., 2019; Motaal et al., 2012; Zaahkoug et al., 2003). The plant's leaf extract is one of the vital parts reported to have lower blood glucose levels in experimentally-induced diabetic rats, as reported by the author's previous work, where it was suggested to have possessed chemicals with antihyperglycemic properties (Mhya et al., 2017). In the light of this, the plant leaf extract was analyzed by GC-MS and found to contain some phenolic compounds like; 2-methoxy phenol (Guaiaicol), 2-methoxy-4-vinylphenol (4 vinylGuaiaicol), 2,6-dimethoxy phenol (Syringol), 2-methoxy-3(-2-propenyl)-phenol (Eugenol), 2-methoxy-4-(1-propenyl)-phenol (Isoeugenol), and 4((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (Mhya et al., 2018).

Literature survey shows that some of these phenolic compounds like; 2-methoxy-3(-2-propenyl)-phenol (Eugenol)

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and 2-methoxy-4-(1-propenyl)-phenol (Isoeugenol) from other medicinal plants act as an inhibitor of alpha-glucosidase (Singh et al., 2016), and as a stimulator of cellular glucose uptake (Kim et al., 2016). However, the modes of action of *B. aegyptiaca* leaf phenolic compounds are mainly unknown. This has prompted the research where in silico drug discovery study was designed to evaluate the potential mechanisms of action of the antihyperglycemic phytochemicals of *B. aegyptiaca* leaf. The in silico study was very effective and significant in modern drug discovery investigations (Pagadala et al., 2008).

2. MATERIALS AND METHODS

2.1. Ligands Preparation

Chemical compounds similarity search was carried out based on the report from a previous study (Mhya et al., 2017), about six phenolic compounds were identified in *B. aegyptiaca* leaves. These phenolic compounds were searched for similarity from the PubChem database, where their details like canonical SMILES PubChem CID etc. were obtained. After downloading the phenolic compounds from PubChem, the 3D structure was used to verify that the structures were stable, optimized conformation. The detailed information for the phenolic compounds was retrieved in SDF files format as (Werner et al., 2006).

The compounds were screened for drug properties and drug activities prediction study using the Predict Activity Spectra of Substances (PASS) online software for possible prediction of various activities related to diabetic management.

2.2. Target Preparation

Following the drug activity prediction study, two proteins that were the important target in the management of diabetes mellitus were identified and then downloaded from protein databank (<http://www.rcsb.org/>). The proteins are; α -amylase (PDB id: 4W93) and α -glycosidase (PDB id: 3TOP). Water molecules were deleted from the original protein, and Utilizing the AutoDock Tool, we introduced polar hydrogen and varied charges as done by Khodade et al. (2007) and saved in a PDBQT file.

2.3. In Silico Bioactivity Analysis

Molecular Docking: Inhibitory potential of phenolics from *B. aegyptiaca* leaves on the enzymes: α -amylase and α -glycosidase were investigated by an in silico molecular docking using AutoDock vina. The docking score with high-affinity energies is generated to select the best interaction pose (Trott & Olson, 2010). The docked conformations and the configuration of the binding pocket residues for target proteins were analyzed using a protein-ligand interaction profiler (PLIP) described by Salentin et al. (2015).

2.4. In Silico Pre-Clinical Testing

ADMET prediction: The prediction of adsorption, distribution, metabolism, excretion and toxicity (ADMET) properties

of the phenolic compounds of *B. aegyptiaca* leaves was done using ADEM-TOX_Drug4, a free ADME-Tox tool as described by Lagorce et al. (2017). Chemical bioactivity scores for therapeutic targets (GPCR ligands, kinase inhibitors, channel modulators, and nuclear receptor modulators) were predicted with Molinspiration Cheminformatics.

Drug Likeness study: Lipinski filter accessible online server was used to ascertain drug-likeness properties of the phenolic compounds based on octanol/water partition coefficient (cLogp), hydrogen bond donors/acceptor and Molar refractivity (Lipinski et al., 2001).

3. RESULTS AND DISCUSSION

3.1. In Silico Similarity Screening of *B. aegyptiaca* Leaf Phenolic Compounds

Table 1 presents the chemical properties such as; Pubchem CID no., molecular formula, molecular weight, Canonical SMILE etc., for the phenolic compounds likely to be from *B. aegyptiaca* leaves following screening from the PubChem database. Meanwhile, pharmacological features including kinase inhibitors, ion channel modulators, and nuclear receptors are shown in Table 2, in which all of the compounds were found to be promising agents. The chemical similarity of phenolics from *B. aegyptiaca* leaf in available chemical databases indicates their existence from other plants and their potential in some biological activities.

Table 3 showed predicted antidiabetic biological activities like insulin promoter, an insulin sensitizer, inhibitor α -amylase and α -glucosidase by the phenolic compounds. All the compounds exert at least one activity where 2-methoxy-4-(1-propenyl)-phenol (Isoeugenol) exhibited all the activities, and 4((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol showed one activity, that is an inhibitor of α -amylase. The compound 2-methoxy phenol (Guaiacol) act as an insulin promoter with a 0.401 probability of active (Pa) and α -amylase inhibitor with Pa at 0.406. 2-methoxy-4-(1-propenyl)-phenol (Isoeugenol) is highest with Pa value at 0.201 as an inhibitor of α -glucosidase. The compound, 2-methoxy-3-(2-propenyl)-phenol (Eugenol), seems to be the most active insulin sensitizer with Pa value at 0.119 and inhibitors of both α -glucosidase and α -amylase.

The portrayed drug activities like insulin promoter, an insulin sensitizer, α -amylase and α -glucosidase inhibition show their attributes in terms of drug-like properties; hence may be good candidates with promising antidiabetic activities. Literature surveys have shown that phenolic compounds; 2-methoxy-3-(2-propenyl)-phenol (Eugenol) from the plant is an effective inhibitor of alpha-glucosidase (Singh et al., 2016). In a recent in-silico study, flavonoid from *Olea europaea* subsp. *europaea* leaves were found to have the potential of inhibiting α -amylase and α -glucosidase activities, among other proteins related to diabetes mellitus Abdelli et al. (2021); Mechchate et al. (2020).

Table 1
Computational Details of *Balanites aegyptiaca* Leaf Phenolic Compounds

S/N	Compounds	PubChem CID	Molecular Formula	Molecular weight (g/mol)	Canonical SMILES
1	2-methoxy phenol (Guaiacol)	460	C ₇ H ₈ O ₂	124.14	COC1=CC=CC=C1O
2	2-methoxy-4-vinylphenol (4 vinylGuaiacol)	332	C ₉ H ₁₀ O ₂	150.17	COC1=C(C=CC(=C1)C=C)O
3	2,6-dimethoxy phenol (Syringol)	7041	C ₈ H ₁₀ O ₃	154.16	COC1=C(C(=CC=C1)OC)O
4	2-methoxy-3-(2-propenyl)-phenol (Eugenol)	596373	C ₁₀ H ₁₂ O ₂	164.20	COC1=C(C=CC=C1O)CC=C
5	2-methoxy-4-(1-propenyl)-phenol (Isoeugenol)	853433	C ₁₀ H ₁₂ O ₂	164.20	CC=CC1=CC(=C(C=C1)O)OC
6	4((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol	1549095	C ₁₀ H ₁₂ O ₃	180.20	COC1=C(C=CC(=C1)C=CCO)O

Table 2
Predicted Drug Properties of *Balanites aegyptiaca* Leaf Phenolic Compounds

S/No.	Compound Name	GPCR Ligand	Ion Channel Modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
1	2-methoxy phenol (Guaiacol)	-2.29	-1.63	-2.30	-2.40	-2.62	-1.75
2	2-methoxy-4-vinylphenol (4 vinylGuaiacol)	-0.96	-0.28	-1.00	-0.77	-1.34	-0.46
3	2-methoxy-4-(1-propenyl)-phenol (Isoeugenol)	-1.02	-0.53	-1.06	-0.70	-1.35	-0.51
4	4((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol	-0.55	-0.05	-0.74	-0.30	-1.00	-0.08

Table 3
Predicted Antidiabetic Activities of *Balanites aegyptiaca* Leaf Phenolic Compounds using Predicted Activity Spectra of Substances (PASS)

S/No.	Compounds	Biological Activities			
		Insulin Promoter	Insulin Sensitizer	α -Amylase Inhibition	α -Glucosidase Inhibition
1	2-methoxy phenol (Guaiacol)	0.401* 0.063**	--	0.406* 0.015**	0.033* 0.012**
2	2-methoxy-4-vinylphenol (4 vinylGuaiacol)	0.269* 0.184**	--	--	0.092* 0.036**
3	2-methoxy-4-(1-propenyl)-phenol (Isoeugenol)	0.297* 0.143**	0.097* 0.050**	0.184* 0.065**	0.201* 0.054**
4	4((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol	--	--	0.271* 0.036**	--

Values with one asterisk (*) indicate Pa (i.e. probability "to be active") while two asterisk (**) indicate Pi (i.e. probability "to be inactive") and -- = absent

3.2. In Silico Bioactivity of *B. aegyptiaca* Leaf Phenolics against α -amylase and α -glucosidase

The binding energies and enzyme's pocket residue amino acids following the docking are presented in Table 4. In contrast, illustrations of enzymes and ligands interactions are shown in Figures 1, 2 a & b. The docking result predicted 2-methoxy-4-(1-propenyl)-phenol (Isoeugenol) from *B. aegyptiaca* leaves interacts with α -amylase with a binding energy of -4.4 kcal/mol against -3.9 kcal/mol by acarbose. While interacting with α -glucosidase, acarbose showed the highest binding energy of -4.8 kcal/mol followed by 2-methoxy-4-(1-propenyl)-phenol (Isoeugenol) which has a binding energy of -4.2kcal/mol. Among the five phenolic compounds from *B. aegyptiaca* leaves extract, the compound; 2-methoxy-4-(1-propenyl)-phenol (Isoeugenol) showed the most potent inhibitor of the two enzymes; α -amylase and α -glucosidase.

Molecular interaction between protein and ligand have been reported to play a vital role in predicting the binding confirmation or pose of the ligand-bound to protein (Pagadala et al., 2016; Trott & Olson, 2010), where the strength of their

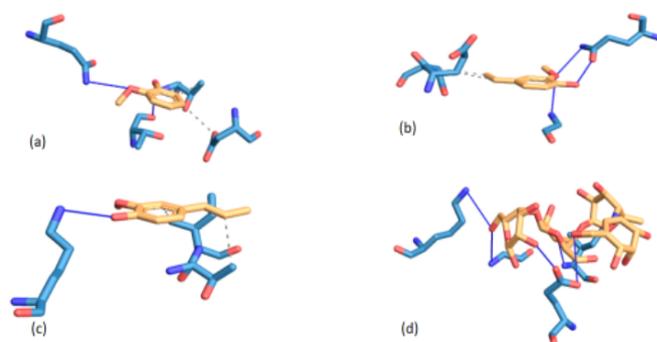


Figure 1. Illustrations of interaction, residue amino acids and binding mode of acarbose and *Balanites aegyptiaca* leaf phenolic compounds with α -amylase Legend: (a) 2-methoxyphenol; (b) 2-methoxy-4-(1-propenyl)-phenol; (c) 4((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol; (d) Acarbose NB: Blue represents amino acids and yellow represents compounds while bold line are hydrogen bonding

Table 4Binding Energy of *Balanites aegyptiaca* Leaf Phenolic Compounds with α -Amylase and α -Glucosidase

Compounds	α -Glucosidase		α -Amylase	
	Binding Energies (kcal/mol)	Ligand-Amino acid interactions	Binding Energies (kcal/mol)	Ligand-Amino acid interactions
2-methoxy phenol (Guaiacol)	-3.8	Thr1101A, Gln1109B, Asp1179A	-3.5	Phe136A, Gly139A, Lys142A
2-methoxy-4-vinylphenol (4 vinylGuaiacol)	-4.0	Gly1102A, Gln1109B, Glu1178A	-	-
2-methoxy-4-(1-propenyl)-phenol (Isoeugenol)	-4.2	Lys1088B, Ile1104A	-4.4	Gly139A, Lys140A, Trp134A, Lys172A
4((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol	-	-	-4.1	Gly139A, Lys140A, Lys172A, Trp134A,
Acarbose	-4.8	Ly1088B, Gly1102A, Ile1104A, Gly1178A	-3.9	Gly139A, Lys140A, Lys142A, Trp134A, Lys172A

NB: - indicates ligand does not dock since it is not an inhibitor of the enzyme based on the predicted activity spectra of substances (PASS) result

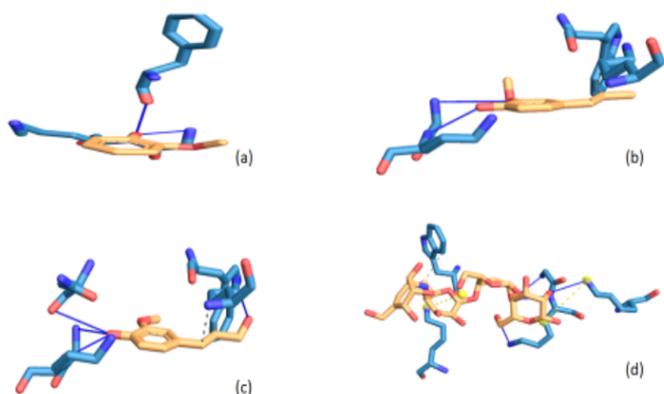


Figure 2. Illustrations of interaction, residue amino acids and binding mode of acarbose and *Balanites aegyptiaca* leaf phenolic compounds with α -glucosidase Legend: (a) 2-methoxyphenol; (b) 2-methoxy-4-vinylphenol; (c) 2-methoxy-4-(1-propenyl)-phenol; (d) Acarbose

NB: Blue represents amino acids and yellow represents compounds while bold line are hydrogen bonding

interactions may be ascertained from their binding energies. Generally speaking, the lower the negative value of total binding energy from the docking score, the more significant the interaction between the proteins and the ligands (Trott & Olson, 2010). Going by this, 2-methoxy-4-(1-propenyl)-phenol (Isoeugenol) interacted strongly with both α -amylase and α -glucosidase as evidenced by its lowest negative binding energies hence, translated as a better inhibitor. Following molecular docking of various phenolic compounds with the enzymes -amylase and -glucosidase, researchers discovered that the compounds with the lowest energy score were the most effective Abdelli et al. (2021).

To justify the potency of the screened compounds, particularly the 2-methoxy-4-(1-propenyl)-phenol (Isoeugenol), which is the most powerful compare to the standard antidiabetic oral drug (Acarbose) in terms of interaction at binding mode. Interestingly, the compound and Acarbose interact with some amino acids like Gly139A, Lys140A, Lys172A and Trp134A in

the active site of α -amylase. By implication, this could explain in part the potency and possible enzyme's inhibitory abilities of the compounds. However, failure to interact with some amino acids like Lys142A in the protein's active site as displayed by Acarbose could be attributable to their small sizes, which may not have appropriately fit in occupying the entire pocket of the active site of α -amylase. In terms of the binding manner of the compounds and acarbose in the active site of -glucosidase, a situation similar to that described above may be applicable..

3.3. In Silico Pre-clinical Testing of *B. aegyptiaca* Leaf Phenolic Compounds

It is shown in Table 5 how well the predicted effects of *B. aegyptiaca* leaves extract on adsorption, distribution, metabolism, excretion, and toxicity have worked out thus far. All the compounds showed a positive response in drug properties for parameters assayed like oral bioavailability, hydrogen donor and acceptor, and water solubility. When the phenolic compounds were studied for drug-likeness properties such as cLogp, hydrogen bond donor/acceptor and Molar refractivity, all six compounds showed a promising result, as presented in Table 6. Where three out of the six compounds showed values that were all within the standard. These compounds are; 2-methoxy-3(-2propenyl)-phenol (Eugenol), 2-methoxy-4-(1-propenyl)-phenol (Isoeugenol) and 4((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol.

For a compound to be deemed "drug-like," it must have a molecular weight of less than 500Da, a number of hydrogen bond donors and acceptors that are fewer than or equal to 10, and a number of hydrogen bond acceptors that are less than or equal to 10. Going by these, one may gladly be stated that the phenolic compounds from *B. aegyptiaca* leaf extract are drug-like. The Lipinski's Rule 5 for drug-likeness of phenolic compounds from *B. aegyptiaca* leaf showed an octanol/water partition coefficient (clogP) that is less than 5, indicating a promising drug candidate. In general, compliance of all the phenolic compounds from *B. aegyptiaca* leaf extract to Lipinski's rule of 5 indicates their drugs like nature.

Table 5
 Predicted Adsorption, Distribution, Metabolism, Excretion and Toxicity of *Balanites aegyptiaca* Leaf Phenolic Compounds

S/No.	Compound Name	Heavy Atom	Hetero Atom	Solubility (mg/L)	Oral (Bioavailability) (EGAN)	Oral (Bioavailability) (VEBER)	Ratio (H/C)	3-75	Status
1	2-methoxy phenol (Guaiaacol)	9	6	7761.53	Good	Good	0.20	Bad	Accepted
2	2-methoxy-4-vinylphenol (4 vinylGuaiaacol)	11	2	10924.28	Good	Good	0.22	Warning	Accepted
3	2-methoxy-4-(1-propenyl)-phenol (Isoeugenol)	12	2	9726.41	Good	Good	0.20	Bad	Accepted
4	4((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol	13	3	22863.16	Good	Good	0.30	Warning	Accepted

Table 6
Lipinski's Rule of 5 for Drug Likeness of *Balanites aegyptiaca* Leaf Phenolic Compounds

S/N	Compounds	PARAMETERS					Molar Refractivity less between 40-130	Status
		MolecularMass (≤ 500 Daltons)	Hydrogen Bond Donor (≤ 5)	Hydrogen Bond Acceptor (≤ 10)	High Lipophilicity Expressed as $\text{LogP} \leq 5$			
1	2-methoxy phenol (Guaiacol)	124.14	1	2	1.227	30.849	Accepted	
2	2-methoxy-4-vinylphenol (4 vinylGuaiacol)	150.18	1	2	1.660	39.370	Accepted	
3	2-methoxy-4-(1-propenyl)-phenol (Isoeugenol)	164.20	1	2	1.904	45.577	Accepted	
4	4((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol	180.20	2	3	1.388	46.450	Accepted	

4. CONCLUSION

In conclusion, the study revealed that phenolic compounds from *B. aegyptiaca* leaf possessed drug-like properties, including interacting with α -amylase and α -glucosidase, important target proteins in the management of diabetes mellitus. The data from the in silico study is a step forward towards the pharmaceutical discovery of antidiabetic drug potential of *B. aegyptiaca* leaf.

CONFLICTS OF INTEREST

The authors have declared that no competing interests.

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AUTHOR CONTRIBUTIONS

All of the authors worked together on this project. DA Mhya developed the study; Ahmad Asmau Nuhu did a literature search; and Mary M. Mankilik drafted the manuscript for the study's final version. The final draft was read and approved by all of the authors.

REFERENCES

- Abdelli, I., Benariba, N., Adjdir, S., Fekhikher, Z., Daoud, I., Said, G., 2021. In silico evaluation of phenolic compounds as inhibitors of α -amylase and α -glucosidase. *Journal of Biomolecular Structure and Dynamics*. 39, 816–822. <https://doi.org/10.1080/07391102.2020.1718553>
- Abubakar, U.S., Abdullahi, S., Ayuba, V., Kaigama, S., Halidu, U.S., Ayuba, M.K., 2017. Medicinal plants used for the management of diabetes mellitus in Zaria, Kaduna state. *Journal of Pharmacy & Pharmacognosy Research*. 5, 156–164.
- Gad, M.Z., El-Sawalhi, M.M., Ismail, M.F., El-Tanbouly, N., 2006. Biochemical study of the anti-diabetic action of the Egyptian plants Fenugreek and Balanites. *Molecular and Cellular Biochemistry*. 281, 173–183. <https://doi.org/10.1007/s11010-006-0996-4>
- Ganesan, K., Rana, M., Sultan, S., 2021. Oral Hypoglycemic Medications. <https://www.ncbi.nlm.nih.gov/books/NBK482386/>
- Guariguata, L., Whiting, D.R., Hambleton, I., Beagley, J., Linnenkamp, U., Shaw, J.E., 2014. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Research and Clinical Practice*. 103, 137–149. <https://doi.org/10.1016/j.diabres.2013.11.002>
- Khodade, P., Prabhu, R., N, C., 2007. Parallel implementation of autodock. *Journal of Applied Crystallography*. 40, 598–599. <https://doi.org/10.1107/S0021889807011053>
- Kim, N., Lee, J.K., Lee, H.J., Lee, Y.W., Kim, H.P., Kim, S.K., 2016. AMPK, a metabolic sensor, is involved in isoeugenol-induced glucose uptake in muscle cells. *The Journal of Endocrinology*. 228, 105–114. <https://dx.doi.org/10.1530/JOE-15-0302>
- Lagorce, D., Bouslama, L., Becot, J., Miteva, M.A., Villoutreix, B.O., 2017. FAF-Drugs4: free ADME-tox filtering computations for chemical biology and early stages drug discovery. *Bioinformatics*. 33, 3658–3660. <https://doi.org/10.1093/bioinformatics/btx491>
- Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*. 6, 3–26. [https://doi.org/10.1016/S0169-409X\(00\)00129-0](https://doi.org/10.1016/S0169-409X(00)00129-0)
- Mechchate, H., Es-Safi, I., Bourhia, M., Kyrilchuk, A., El-Moussaoui, A., Boust, D., 2020. In-Vivo Antidiabetic Activity and In-Silico Mode of Action of LC/MS-MS Identified Flavonoids in Oleaster Leaves. *Molecules*. 25, 5073. <https://doi.org/10.3390/molecules25215073>
- Mhya, D.H., Anigo, A.J.O., Umar, K.M., A, I., 2019. Effect of Fractions of Balanites aegyptiaca Leaf Extracts on the Activities of Glucose Metabolizing Enzymes in Diabetic Rats. *Biomed Eng & Sci*. 6, 11–22.
- Mhya, D.H., Anigo, K.M., Umar, I.A., Alegbejo, J.O., 2017. Evaluation of phytoconstituent of Balanites aegyptiaca (l) del leaves and fruit-mesocarp extracts. *MOJ Bioorganic & Organic Chemistry*. 1, 228–232. <https://doi.org/10.15406/mojboc.2017.01.00039>
- Mhya, D.H., Anigo, K.M., Umar, I.A., Alegbejo, J.O., 2018. Anti-hyperglycemic effect of balanites aegyptiaca leaves extract-fractions in streptozotocin-induced diabetic rats. *Journal of Complementary and Alternative Medical Research*. 6, 1-12. <https://doi.org/10.9734/JOCAMR/2018/41997>
- Mirza, A.Z., Arayne, M.S., Sultana, N., Qureshi, F., 2013. Spectroscopic study to characterize in vitro interaction of losartan with gliquidone and pioglitazone. *Medicinal Chemistry Research*. 22, 351–359. <https://doi.org/10.1007/s00044-012-0036-8>
- Motaal, A.A., Shaker, S., Haddad, P.S., 2012. Antidiabetic activity of standardized extracts of Balanites aegyptiaca fruits using cell-based bioassays. *Pharmacognosy Journal*. 4, 20–24. <https://doi.org/10.5530/pj.2012.30.4>
- Pagadala, N.S., Syed, K., Tsuzynski, J., 2016. Software for molecular docking: a review. *Biophysical Reviews*. 9, 91–102. <https://doi.org/10.1007/s12551-016-0247-1>
- Salentin, S., Schreiber, S., Haupt, V.J., Adasme, M.F., Michael, M.S., 2015. PLIP: fully automated protein-ligand interaction profiler. *Nucleic Acids Research*. 43, 443–447. <https://dx.doi.org/10.1093/nar/gkv315>
- Singh, P., Jayaramaiah, H.R., Agawane, S.B., Vannuruswamy, G., Korwar, A.M., Anand, A., 2016. Potential Dual Role of Eugenol in Inhibiting Advanced Glycation End Products in Diabetes: Proteomic and Mechanistic Insights. *Scientific Reports*. 6, 18798. <https://doi.org/10.1038/srep18798>
- Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multi-threading. *Journal of Computational Chemistry*. 31, 455–461. <https://dx.doi.org/10.1002/jcc.21334>
- Werner, J.G., Kevin, E.G., Mark, W., Cornelis, D.V., V, J., 2006. Optimizing the use of open source software applications in drug discovery. *Drug Discovery Today*. 11, 127–132. [https://doi.org/10.1016/S1359-6446\(05\)03692-5](https://doi.org/10.1016/S1359-6446(05)03692-5)
- Zaahkouk, S.A., Rashid, S.Z., Mattar, A.F., 2003. Anti-diabetic properties of water and ethanolic extracts of Balanites aegyptiaca fruits flesh in senile diabetic rats. *The Egyptian Journal of Hospital Medicine*. 10, 90–108. <https://dx.doi.org/10.21608/ejhm.2003.18736>