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In-silico pharmacokinetics study of phenolic compounds identified from *Ageratum conyzoides*

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The extent of drug action is in direct relationship with the amount of the drug in aqueous medium in contact with the substrate molecules. The factors affecting this concentration in a biological system can be classified into the pharmacokinetics (PK) phase and the pharmacodynamics phase of drug action. Thus, this research focused on the *in silico* PKs analyses of phenolics (furocoumarinic acid, liquiritin, isorhamnetin and syringin) identified from ethylacetate fraction of methanol leaf extract of *A. conyzoides*. Determination of the pharmacokinetics and physicochemical properties of the above phenolics were achieved using SwissADME, ADMETlab 2.0 and SuperCYPsPred webservers synergistically. The phenolics with best in-silico pharmacokinetics parameters was further studied experimentally using *in-vitro* α-amylase and α-glucosidase inhibition assays. The findings indicated that four phenolics are soluble in water, and all the ligands have consensus logP values less than 5 according to Lipinski's Rule of 5, with isorhamnetin being the best (Log*P* 1.6). Also, most of the phenolics are non-inhibitors of the main CYP450 isozymes, but 1A2 and 3A4 were inhibited by isorhamnetin. Similarly, they are mostly non-substrates of the isozymes, but 2C9, 2C19, and 2D6 were metabolized by isorhamnetin. Inhibition assays using isorhamnetin-containing sample indicated that the inhibitory effects were more on α-glucosidase (IC_{50} of 18.11 and 15.97µg/ml for acarbose and isorhamnetin, respectively) than on α-amylase. This study has demonstrated that these phenolics from ethylacetate fraction of methanol leaf extract of *A. conyzoides* have relatively good pharmacokinetics within the acceptable limit of drug-like molecules.

Keywords: *pharmacokinetics, in-silico, Ageratum conyzoides, phenolics, ethylacetate*

Introduction

A wide range of experimental technologies providing insight into the fate of drugs account for the essential role of drug metabolism in drug discovery and development. Numerous computational techniques have been developed to predict the metabolic fate of drug candidates due to the high expense of traditional drug development processes. This has made it possible to screen a huge number of molecules with concomitant identification of relatively small amount of viable ones (Kazmi et al., 2019). Many experimental tools have been used in the last few decades to investigate the metabolism and destiny of pharmaceuticals (Shadid et al., 2018; Diao et al., 2017). The extent of drug action is in direct relationship with the quantities of the molecule in aqueous environment in contact with the substrate molecules. The factors influencing this concentration in a biological system can be classified into the PKs phase and the pharmacodynamics phase of drug activity. The pharmacodynamics phase examines the chemical nature of the interaction between the drug and its target, or the influence of the drug on the body, whereas the pharmacokinetic phase studies the parameters that govern the drug's path from its site of administration to its site of action (Li et al., 2016). Due to some advantages which include lower costs the use of *in-silico*, or computerized research, in drug design and development is becoming increasingly clear and

important. Virtual screening, also known as *in-silico* screening, is a methodical process for selecting the Launchpad for a computer-aided drug design (CADD) campaign (Talevi, 2016; Zhu et al., 2013). Computer-aided drug discovery is the method or technique that has the least technological gap between high- and low-income countries, and has been in use in drug discovery cycle. Consequently, by subjecting recently discovered drug candidates to ADME (absorption, distribution, metabolism, and excretion) properties analysis and prediction, it can aid in the elimination of compounds with unacceptable druggability features. The physiochemical properties and structural characteristics of already existing drug molecules and potential candidate drugs, has been extremely used to filter or separate out compounds with questionable properties, especially poor ADME profiles. The pharmacokinetics property analysis and forecasting of drug candidates can be performed using *in-silico* programs like ADMETLab 2.0 and SwissADME (Adel et al., 2023; Shaaban et al., 2023). Furthermore, drug metabolism is crucial for both medication bioavailability and drug-drug interactions (DDIs) (Palleria et al., 2013). Predicting if the ligands under study will likely be substrates or inhibitors of significant PK-related proteins such as permeability glycoprotein (P-gp) and cytochrome P450 (CYP) is therefore essential. Approximately two-thirds of all known drugs in humans are metabolized by the 57 isozymes that constitute the human cytochrome P450 family (phase I enzymes). Of these, 80% to 90% are assigned to five isozymes: the CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 (Di, 2014). One of the most important sources of medicine from the beginning of human civilization has been plants. Medicinal plants are in abundance of pharmaceutical chemicals that are used in illness prevention and treatment. The annual herbaceous plant, *A. conyzoides* L. has been utilized traditionally for a very long time to treat or manage a wide range of conditions, including high blood sugar, toothaches, pneumonia, and many more. Consequently, a wide range of phytoconstituents from almost every part of this plant have been studied to determine their potential therapeutic value, including alkaloids, flavonoids, terpenes, and sterols (Yadav et al., 2019; Ashande et al., 2015). These phytoconstituents have demonstrated a varied number of pharmacological actions, such as antimicrobial, anti-inflammatory, analgesic, antioxidant, anti-cancer, anti-protozoal, and antidiabetic (Ozioko et al., 2022; Rahman 2012). Thus, the *in-silico* assessment and analysis of PKs of phenolics, which were previously identified by Ozioko et al. (2024) from ethylacetate fraction of *A. conyzoides* methanol leaf extract, was the main focus of this study in quest for novel oral antidiabetic drug discovery.

Materials and Methods

All reagents were of analytical grade. Materials and reagents used include *A. conyzoides* leaves, 0.5M Tris–HCl buffer at pH 6.9, 0.01M $CaCl_2$, starch, 50% acetic acid, 0.9% normal saline, 1% glucose, 5mg acarbose, 1M 4-nitrophenyl-1-β-D-glucopyranoside (pNPG) and 0.1N Na_2CO_3. Collection and Extraction of *A. conyzoides* leaves was conducted according to Ozioko et al. (2022).

Ligands

The ligands in this study were the phenolics identified by Ozioko et al. (2024) from ethylacetate fraction of methanol leaf extract of *A. conyzoides*, and some US FDA approved oral hypoglycemic drugs. They were downloaded and retrieved from the PubChem database (Kim et al., 2016) of National Center for Biotechnology Information (NCBI) (https://pubchem.ncbi.nlm.nih.gov) (Table 1). Each anti-diabetic class was represented with an example.

Table 1. The Chemical Formula and PubChem CID of Ligands used in this Study

S/No	Ligands	PubChem CID	Molecular Formula	Anti-diabetic Class
1	Glibenclamide	3488	$C_{23}H_{28}ClN_3O_5S$	Sulfonylurea
2	Metformin	4091	$C_4H_{11}N_5$	Biguanides
3	Rosiglitazone	77999	$C_{18}H_{19}N_3O_3S$	Thiazolidinediones(TZD)
4	Acarbose	41774	$C_{25}H_{43}NO_{18}$	α-Glucosidase Inhibitors
5	Sitagliptin	4369359	$C_{16}H_{15}F_6N_5O$	DPP-4 Inhibitor
6	Repaglinide	65981	$C_{27}H_{36}N_2O_4$	Meglitinides
7	Furocoumarinic acid	31750885	$C_{17}H_{18}O_9$	-
8	Liquiritin	503737	$C_{21}H_{22}O_9$	-
9	Isorhamnetin	5281654	$C_{16}H_{12}O_7$	-
10	Syringin	5316860	$C_{17}H_{24}O_9$	-

DPP= Dipeptidyl peptidase-4.

Pharmacokinetics and physicochemical properties of the ligands

The prediction of pharmacokinetics and the physicochemical properties of the identified phenolic compounds and some oral hypoglycemic drugs were carried out using SwissADME online tool (Daina et al., 2017) (http://www.swissadme.ch)

and the ADMETlab 2.0 tool (Xiong et al., 2021). Similarly, the prediction of effects of the ligands on most common CYP450 drug metabolizing enzymes was carried out according to the online tools, SwissADME, SuperCYPsPred (Banerjee et al., 2020) http://insilico-cyp.charite.de/SuperCYPsPred/) and the ADMETlab 2.0 tool (Xiong et al., 2021). Understanding which medications function as substrates, inducers, or inhibitors of the implicated enzymes might help avoid interactions that are clinically insignificant. The present study concentrated on five key isoforms of CYP450, as previous research has indicated that 50 to 90% of therapeutic compounds are either substrates or inhibitors of these five isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) (Di, 2014). These tools were equally used to evaluate the ligands effect on permeability glycoprotein (Pg-protein).

In-vitro α-Amylase and α-glucosidase inhibition assays of the fraction containing phenolics with the best PKs

The ethylacetate fraction of methanol leaf extract of *A. conyzoides* containing the phenolic compound with relatively best pharmacokinetic properties were validated experimentally using *in-vitro* inhibition activity assays. Thus, isorhamnetin-containing fraction or sample was used for this purpose.

In-vitro α-amylase inhibitory activities of isorhamnetin-containing sample

This experiment was conducted based on the protocol reported by Tamil et al. (2010).

Principle: Alpha-amylase catalyzes carbohydrate breakdown by hydrolysis of internal 1, 4-β-glycosidic linkages of polysaccharides (starch, glycogen) to disaccharides. Alpha-glucosidase then catalyzes the conversion of the disaccharides to monosaccharides, which increases the postprandial hyperglycemia. The hydrolysis of the glycosidic bond is facilitated by an acid hydrolysis mechanism, that is facilitated by Asp197 and Glu233 in porcine pancreatic amylase.

Preparation of substrate solution: This was conducted according to Tamil et al. (2010).

Experimental procedure: Isorhamnetin-containing sample was dissolved and prepared at various concentrations of 10, 25, 50, 75, 100, 125, 150, 175, and 200µg/ml using 0.9% normal saline. The test tube holding the substrate solution was then filled with the sample solution (0.2 ml) at various concentrations. Each combination then received an addition of porcine pancreatic amylase (0.1 ml in Tris–HCl buffer (2 units/ml). After 10 minutes at 37°C, 0.5ml of 50% acetic acid was added to each test tube to halt the reaction. After centrifuging the mixture for 5 minutes at 4ºC at 3000 rpm, the supernatants were collected, and the absorbance measured with spectrophotometer at 595nm. Acarbose, an α-amylase inhibitor, was employed as a positive control in this experiment. The protocol was carried out in triplicate for each concentration.

To estimate the α-amylase inhibitory activity, this formula was used:

$$\text{Inhibitory activity of extract} = [(AC - AS)/AC] \times 100$$

AC is the absorbance of the control (100% enzyme activity) and AS is the absorbance of the samples. Control represents 100% enzyme activity and was carried out in a similar fashion by substituting isorhamnetin sample with distilled water. With respect to blank, the enzyme solution was only substituted with distilled water. Percentage inhibition then plotted against the sample concentration. From the plot, the concentration of the isorhamnetin-containing sample or acarbose resulting in 50% enzyme inhibition (IC_{50}) were determined.

In-vitro α-glucosidase inhibitory activities of isorhamnetin-containing sample

This assay was conducted based on the protocol of Pistia-Brueggeman & Hollingsworth (2001).

Principle: Alpha-glucosidases are enzymes in the digestive tract that hydrolyze carbohydrate into glucose units. It hydrolyzes the substrate mixture to release the p-nitrophenol that can be measured colorimetrically at 405nm. Preparation of substrate solution: A 1.0M 4-nitrophenyl-1-β-D-glucopyranoside solution was prepared.

Experimental procedure: The isorhamnetin-containing sample was dissolved in 0.9% normal saline, and 50µl was made at various concentrations of 10, 25, 50, 75, 100, 125, 150, 175, and 200µg/ml. A mixture of 10µl α-glucosidase (maltase) 1U/ml and 125µl of 0.1M phosphate buffer (pH 6.8) was then incubated for 20 minutes at 37ºC. A solution of 20µl of 1.0M 4-nitrophenyl-1-β-D-glucopyranoside (pNPG) substrate was added, and the reaction was then allowed to proceed for 30 minutes. Then 50µl of 0.1M Na_2CO_3 was injected to terminate the reaction. At 405nm, the absorbance was

determined with a spectrophotometer. Acarbose (α-glucosidase inhibitor) was used as positive control of the experiment. For every concentration, the assay was conducted triplicate.

To calculate the α-glucosidase inhibitory activity, this formula was used:

$$\text{Extract inhibitory activity} = [(AC - AS)/AC] \times 100$$

AC is the absorbance of the control and AS is the absorbance of the samples. Control represents 100% enzyme action and was carried out in a similar fashion by substituting isorhamnetin sample with pure water. With respect to blank, we substituted the enzyme solution with distilled water. Percentage inhibition then plotted against the sample concentration. From the plot, the concentration of the isorhamnetin sample or acarbose resulting in 50% enzyme inhibition (IC_{50}) were determined.

Results

Figure 1 presented the 2-D chemical structures of the phenolics identified from ethylacetate fraction of *A. conyzoides* methanol leaf extract drawn using ChemDraw Pro 12 whose PKs were investigated computationally. The chemical structures showed that furocoumarinic acid is a phenolic glycoside, liquiritin- polyphenol, isorhamnetin- flavonol, and syringin- also phenolic glycosides

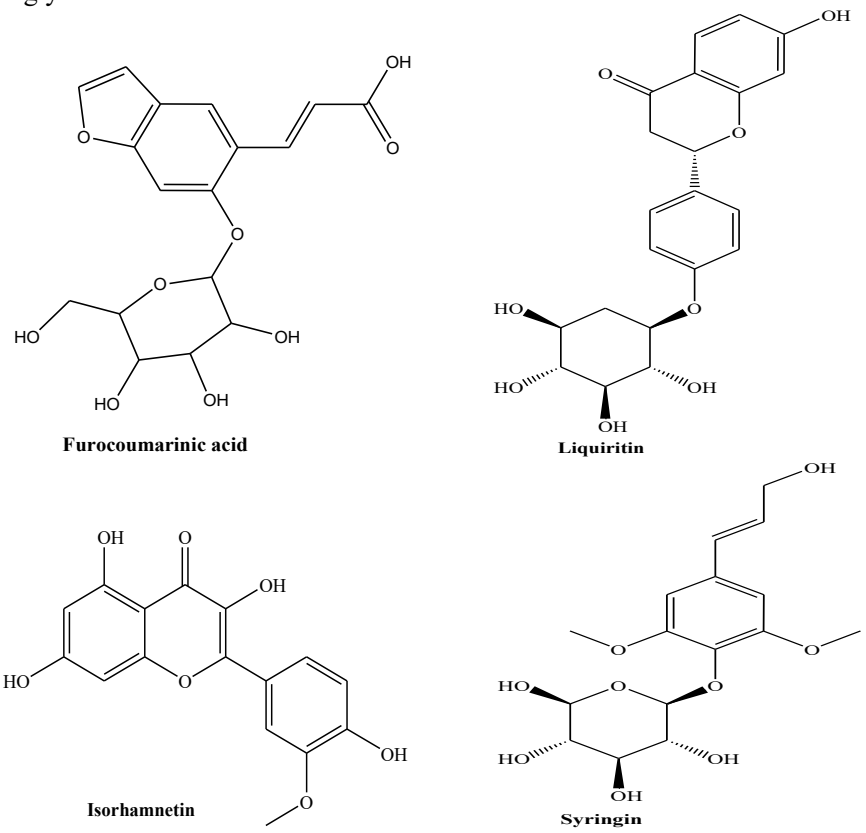


Figure 1. 2-D Structures of the identified phenolics from ethylacetate fraction drawn using ChemDraw

The general physicochemical properties of the ligands

Table 2 indicated the predicted physicochemical properties of the ligands which comprises the water solubility, lipophilicity, molecular weight (MW), molar refractivity (MR), topological polar surface area (TPSA), numbers of hydrogen bond acceptors and donors, and number of rotatable bonds. The result indicated that all the ligands are soluble, but acarbose and metformin are highly soluble. The result of the lipophilicity indicated that the respective values for each ligand was within the Lipinski Rule of 5 (Ro5) (LogP<5) with isorhamnetin having the most ideal value. As indicated, acarbose is the least soluble in organic medium (high negative LogP value). Also, only acarbose violated the Lipinski Ro5 with respect to MW (MW>500 g/mol) as well as numbers of hydrogen bonds acceptor and donor. As for No RB, only glibenclamide and repaglinide had values above the ideal value of ≤9.

Table 2. The predicted general physicochemical properties of the ligands

Ligands	Physicochemical Properties										
	MW (g/mol)	F Csp 3	No RB	No HBA	No HBD	MR	TPSA(Å^2)	Water Solubility Log S	Water Solubility Class	Lipophilicity CLogP	Lipophilicity LogD7.5
Glibenclamide[4]	494.01	0.39	**11.00**	5.00	3.00	126.25	121.98	-5.47	M soluble	3.57	1.75
Metformin[1]	129.16	0.50	2.00	2.00	3.00	36.93	91.49	0.30	H Soluble	-0.91	-2.06
Rosiglitazone[3]	357.43	0.81	7.00	4.00	3.00	101.63	96.83	-3.82	Soluble	2.38	3.01
Acarbose[1]	**645.60**	0.92	9.00	**19.00**	**14.00**	136.69	**321.17**	2.58	H Soluble	-6.23	-2.44
Sitagliptin[3]	407.31	0.55	6.00	10.00	1.00	87.25	77.04	-2.72	Soluble	2.50	1.81
Repaglinide[4]	452.59	0.48	**11.00**	4.00	2.00	135.45	78.87	-5.46	M Soluble	4.52	4.97
Furocoumarinic acid[3]	366.32	0.35	5.00	9.00	5.00	87.03	**149.82**	-2.13	Soluble	-0.06	0.85
Liquiritin[3]	418.39	0.38	4.00	9.00	5.00	101.69	**145.91**	-2.73	Soluble	0.43	1.26
Isorhamnetin[3]	316.26	**0.06**	2.00	7.00	4.00	82.50	120.36	-3.39	Soluble	1.66	2.25
Syringin[2]	372.37	.035	7.00	9.00	5.00	89.63	138.07	-1.04	V Soluble	-0.69	-0.03

All parameters were predicted using SwissADME tool but LogD7.4, which was predicted with ADMETlab 2.0.
MW= Molecular weight; F= fraction; No RB= Number of rotatable bonds; No HBA= Number of hydrogen bond acceptors; No of HBD= Number of hydrogen bond donors; MR= molecular refractivity; TPSA= Topological polar surface area; H= highly; M= moderately; log *S*= molar solubility in water; Ac= acid; C=consensus; and LogD7.4=lipophilicity at pH 7.4.

The pharmacokinetics studies of the ligands

The pharmacokinetics properties which comprises the absorption, distribution, metabolism, and excretion (ADME) were as presented in Tables 3 to 7. Table 3 presented the results of different properties which influences and affects the absorption of oral drugs. The values for HIA (human intestinal absorption) and human oral bioavailability (F 20% and 30%) were categorical, in that 1 and 0 connote the probability of been positive and negative respectively. Out of the 4 phenolics identified, only isorhamnetin had high probability of intestinal or gastrointestinal absorption. Also, all the ligands had high probability of F (20%). However, the values for MDCK (Madin−Darby Canine Kidney cells) and Caco-2 (human colon adenocarcinoma cell lines) permeabilities were absolute. Only isorhamnetin had ideal value for Caco-2 permeability among the 4 phenolics. However, all the ligands were within the ideal values for MDCK permeability.

Table 3. Predicted absorption of the ligands

Ligands	Parameters						
	HIA	GIA	F(20%)	F(30%)	Caco-Perm (cm/s)	MDCK Perm (cm/s)	Log K_p (cm/s)
Glibenclamide	1.00	Low	1.00	1.00	-5.50	4.71×10^{-5}	-5.90
Metformin	1.00	High	1.00	1.00	-6.26	1.95×10^{-3}	-7.99
Rosiglitazone	1.00	High	1.00	1.00	**-4.88**	2.77×10^{-5}	-6.27
Acarbose	0.00	Low	0.00	0.00	-6.15	8.9×10^{-4}	-6.27
Sitagliptin	1.00	High	1.00	1.00	**-5.07**	1.43×10^{-5}	-8.29
Repaglinide	1.00	High	1.00	1.00	**-4.87**	2.27×10^{-5}	-5.38
Furocoumarinic acid	0.00	Low	1.00	0.00	-5.83	4.85×10^{-5}	-8.45
Liquiritin	0.00	Low	1.00	0.00	-6.19	2.94×10^{-5}	-8.58
Isorhamnetin	1.00	High	1.00	0.00	**-5.06**	9.45×10^{-6}	-6.90
Syringin	0.00	Low	1.00	0.00	-5.45	1.74×10^{-4}	-9.50

HIA= human intestinal absorption; GIA= gastrointestinal absorption; MDCK= Madin−Darby Canine Kidney cells; Caco-2= human colon adenocarcinoma cell lines; Perm= permeability; F(20%)= 20% human oral bioavailability; F(30%)= 30% human oral bioavailability; and K_p =skin permeability coefficient.

Note that 1 and 0 are categorical values which connote the probability of being positive and negative respectively. A molecule is said to have a high passive MDCK permeability for a P*app* > 20×10^{-6} cm/s, moderate permeability for 2-

$20x10^{-6}$cm/s, low permeability for < $2x10^{-6}$cm/s. Also, a molecule is said to have a good Caco-2 permeability if it has predicted value >-5.15log cm/s. For skin permeability, the negative value of the log K_p increases, the less skin permeant the ligand becomes. All parameters were predicted by ADMETLab 2.0 tool, except GIA and Log*Kp* that was predicted by SwissADME.

Table 4 presented the results of the factors that influence oral drug distribution and excretion. Plasma protein binding (PPP), blood-brain barrier (BBB), volume of distribution (VD) and fraction unbound (Fu) were factors that affect drug distribution, while time and clearance were the factors that influence drug excretion that were predicted. With respect to BBB, 1(Yes) and 0 (No) are categorical values which connote the high probability of BBB permeant and non-permeant respectively. The result indicated that most of the ligands were non-permeant. Also a compound is considered to have a proper PPB if it has predicted value < 90%, and is considered to have a proper VD if it has predicted VD in the range of 0.04-20L/kg. All the VD values were within this range.

Table 4. Predicted distribution and excretion of the ligands

Ligands	Parameters						
	PPB(%)	BBB1	BBB2	VD(L/kg)	Fu(%)	$T^1/_2$(hr)	CL (mL/min/kg)
Glibenclamide	90.81	0.00	No	0.34	0.75	1.13	0.62
Metformin	23.53	0.00	No	1.16	74.15	1.84	3.50
Rosiglitazone	93.66	0.00	No	0.48	3.16	1.38	8.79
Acarbose	21.14	1.00	No	0.07	62.88	1.32	0.37
Sitagliptin	74.85	0.00	Yes	2.94	58.64	1.22	6.16
Repaglinide	95.15	0.00	No	0.21	1.33	1.69	3.09
Furocoumarinic acid	57.58	0.00	No	0.39	20.15	0.68	1.80
Liquiritin	80.12	0.00	No	0.45	19.50	1.04	4.42
Isorhamnetin	90.71	0.00	No	0.65	8.51	0.66	6.99
Syringin	43.40	0.00	No	0.55	42.02	0.99	2.40

PPB= plasma protein binding; BBB= blood brain barrier; VD= volume distribution; Fu=fraction unbound in plasm; $T^1/_2$= half-life; CL= clearance. The BBB1 and 2 were predicted using ADMETLab 2.0 and SwissADME tools respectively. All other parameters were predicted using ADMETLab 2.0. Note that 1 and 0 are categorical values which connote the probability of being positive and negative respectively. A molecule is said to have a good PPB if it has predicted value < 90%. Also, a potential drug candidate is said to have a good VD if it has predicted VD in the range of 0.04-20L/kg. The predicted Fu is interpreted as follows: >20%- High Fu; 5-20%- medium Fu; <5% low Fu. Also, the predicted CL result interpretation is as follows: >15 ml/min/kg- high clearance; 5-15ml/min/kg- moderate clearance; <5 ml/min/kg- low clearance.

The result of Table 5 showed the potential inhibitory effects of the ligands on the five main CYP450 isozymes as predicted using SwissADME, ADMETLab 2.0 and SuperCYPsPred. SwissADME tool model returned "Yes" or "No" if the ligand under investigation has higher probability of been an inhibitor or non-inhibitor respectively of a given CYP, just as in SuperCYPsPred web server prediction model. However, ADMETLab 2.0 model returned respectively "1" or "0" if the ligand has higher probability of been an inhibitor or non-inhibitor of a given CYP. From the result (Table 5), most of the ligands were found to follow similar pattern when the outputs from the three web servers were juxtaposed together, except for rosiglitazone and repaglinide. For instance, glibenclamide was predicted to be inhibitors of all the CYP isozyme except for 2C19 (from ADMETLab 2.0 only). Interestingly, metformin, acarbose, furocoumarinic acid, liquiritin, and syringin (except for 2C9 in SuperCYPsPred) were predicted to be non-inhibitors of all the CYP isozymes. However, isorhamnetin was found to be inhibitors of 1A2 and 3A4 from the three tools and non-inhibitors of 2C9, 2C19 and 2D6.

Table 5. Predicted inhibition potential of the ligands on CYP450 isozymes

Ligands	P450 Isoforms														
	SuperCYPsPred					SwissADME					ADMETLab 2.0				
	1A2	3A4	2C9	2C19	2D6	1A2	3A4	2C9	2C19	2D6	1A2	3A4	2C9	2C19	2D6
Glibenclamide	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	0	1	1	0	1
Metformin	No	No	No	No	No	No	No	No	No	No	0	0	0	0	0
Rosiglitazone	No	Yes	No	No	No	No	Yes	Yes	Yes	Yes	1	1	1	1	1
Acarbose	No	No	No	No	No	No	No	No	No	No	0	0	0	0	0
Sitagliptin	No	No	No	No	No	No	No	No	No	No	0	0	0	0	0
Repaglinide	No	Yes	No	No	No	No	Yes	No	Yes	Yes	0	1	1	1	0

Furocoumarinic acid	No	No	No	No	No	No	No	No	No	No	0	0	0	0	0
Liquiritin	No	No	No	No	No	No	No	No	No	No	0	0	0	0	0
Isorhamnetin	Yes	Yes	No	No	No	Yes	Yes	No	No	Yes	1	1	0	0	1
Syringin	No	No	Yes	No	No	No	No	No	No	No	0	0	0	0	0

On the other hand, Table 6 presented the results of potential substrate effects of the ligands on the five main CYP isozymes using SuperCYPsPred and ADMETLab 2.0 tools because the SwissADME tool had no interface for substrate prediction. From the result, glibenclamide was found to be substrate for all the isoforms except for 2D6 and 1A2 (only in ADMETLab 2.0). Metformin showed variations in 2C19 and 2D6, while acarbose showed only variation in 2C19 (as substrate) and as non-substrate for other isoforms. More so, furocoumarinic acid was predicted to be non-substrates but for 2C9 (in ADMETLab 2.0), while liquiritin was found to be substrate for only 2C9 and 2D6 of ADMETLab 2.0. Similarly, syringin was found to be non-substrates for all the isoforms using both tools. However, isorhamnetin was predicted not to be metabolized by 1A2 and 3A4 using SuperCYPsPred and 3A4 only for ADMETLab 2.0.

Table 6. Predicted substrate potentials of the ligands on CYP450 isozymes

Ligands	**P450 Isoforms**									
	SuperCYPsPred					**ADMETLab 2.0**				
	1A2	**3A4**	**2C9**	**2C19**	**2D6**	**1A4**	**2A4**	**2C9**	**2C19**	**2D6**
Glibenclamide	Yes	Yes	Yes	Yes	No	0	1	1	1	0
Metformin	Yes	No	No	No	Yes	0	0	0	1	1
Rosiglitazone	Yes	No	Yes	Yes	No	1	0	1	0	1
Acarbose	No	No	No	No	No	0	0	0	1	0
Sitagliptin	No	Yes	No	No	No	0	1	0	0	1
Repaglinide	Yes	Yes	Yes	Yes	No	1	1	1	1	0
Furocoumarinic acid	No	No	No	No	No	0	0	1	0	0
Liquiritin	No	No	No	No	No	0	0	1	0	1
Isorhamnetin	No	No	Yes	Yes	Yes	1	0	1	1	1
Syringin	No	No	No	No	No	0	0	0	0	0

"Yes" or "1" and "No" or "0" connote that a ligand is an inhibitor and non-inhibitor respectively.

The potential effects of the ligands on permeability glycoprotein (P-gp) were as recorded in Table 7. The ADMETLab 2.0 and SwissADME tools were used to predict these effects. SwissADME only showed result whether the ligand is substrate ("Yes") or non-substrate ("No"), while ADMETLab 2.0 showed for both inhibitory and substrate effects of the ligands. Comparing the results from the two tools, it was only acarbose that was predicted to be substrate, while rosiglitazone, furocoumarinic acid, isorhamnetin and syringin were non-substrates to P-gp using both tools. The rest of the ligands respectively showed opposite effect. As for the inhibitory effects, all the ligands were found to be non-inhibitors ("0") of P-gp but glibenclamide.

Table 7. Predicted effect of the ligands on the permeability glycoprotein (P-gp)

Ligands	**P450 Isoforms**		
	SuperCYPsPred		**ADMETLab 2.0**
	P-gp Inhibitors	**P-gp Substrates**	**P-gp Substrates**
Glibenclamide	1	1	No
Metformin	0	1	No
Rosiglitazone	0	0	No
Acarbose	0	1	Yes
Sitagliptin	0	0	Yes
Repaglinide	0	0	Yes
Furocoumarinic acid	0	0	No
Liquiritin	0	0	Yes
Isorhamnetin	0	0	No
Syringin	0	0	No

P-gp= permeability glycoprotein.

Table 8. *In-vitro* inhibition of α-amylase activity by isorhamnetin-containing sample

Sample Conc. (µg/ml)	Percentage Inhibition (%)	
	Acarbose	Isorhamnetin-containing Sample
10.00	45.13±1.08	51.86±0.64
25.00	43.33±0.31	61.05±0.78
50.00	77.96±0.92	78.1±0.51
75.00	83.02±1.46	81.38±1.13
100.00	91.92±0.32	90.35±0.94
125.00	92.61±0.74	91.99±0.41
IC_{50}	50.33(µg/ml)	54.42(µg/ml)

The values were presented as Mean±SD. A Two-sample t-test analysis indicated that there is no statistically significant difference between the mean % inhibition of acarbose and isorhamnetin, with t(df) = 0.42 (9), and a P-value = 0.340 (P>0.05).

Tables 8 and 9 present the results of *in-vitro* inhibition of α-amylase and α-glucosidase activities by isorhamnetin-containing sample. The ADME properties analyses indicated that isorhamnetin relatively exhibited best drug-like properties with high intestinal absorption and bioavailability.

Table 8 showed the result of *in-vitro* inhibitions of α-amylase activity by isorhamnetin-containing sample. According to the result, the % inhibition by both the acarbose (standard) and isorhamnetin-containing samples were concentration dependent. Reaching a concentration of 100 µg/ml upwards, the enzyme tends to be saturated resulting in approximately equal % inhibition. However, the IC_{50} value of acarbose (50.33 µg/ml) was slightly better than that of isorhamnetin (54.42 µg/ml), but no statistical significant difference between the mean % inhibition of acarbose and isorhamnetin (P<0.05). Table 9 on the other hand presented the result of α-glucosidase inhibitory activities by acarbose and isorhamnetin-containing sample. The result displayed similar pattern as that of α-amylase but with higher % inhibition values. However, the isorhamnetin IC_{50} value (15.97µg/ml) was lower and better than that of acarbose (18.11µg/ml). Despite the differences in inhibitors concentrations at 50% enzyme inhibition, there was no statistical significant difference between the mean % inhibition of acarbose and isorhamnetin (P<0.05).

Table 9. *In-vitro* inhibition of α-glucosidase activity by isorhamnetin-containing sample

Sample Conc. (µg/ml)	Percentage inhibition (%)	
	Acarbose	Isorhamnetin-containing sample
10.00	65.23±1.66	66.27±0.90
25.00	72.19±1.11	73.03±0.60
50.00	80.05±1.07	80.05±1.07
75.00	85.06±0.79	81.29±0.77
100.00	92.74±1.07	89.63±0.69
125.00	92.05±1.27	90.83±0.79
IC_{50}	18.11(µg/ml)	15.97(µg/ml)

The values were presented as Mean±SD. A Two-Sample t-Test analysis indicated that there is no statistical significant difference between the mean % inhibition of acarbose and isorhamnetin with t(df)= -0.26(10) and P-value = 0.601 (P<0.05).

Discussion

The amount of drug in the aqueous media in close interaction with the substrates directly affects the degree of drug action. Similarly, the solubility of a drug is one of the important variables influencing its distribution and absorption, and is very important for oral drug administration and development (Ottaviani et al., 2010). The results in Table 2 showed the ligands' water solubility, lipophilicity, and other physicochemical characteristics. High solubility is necessary for some medications in order to supply a sufficient amount of the active ingredient. The ligands denoted by superscripts 1, 2, 3, and 4 are grouped as highly soluble, very soluble, soluble, and moderately soluble, in that order, as presented in the result (Table 2). Hence, the four identified phenolics can be said to be soluble, with metformin and acarbose being the most highly soluble in water. Most drug development operations are considerably influenced by the presence of a soluble substances, particularly in terms of ease of processing and formulation (Ritchie et al., 2013). However, the partition coefficient, also known as lipophilicity ($P_{o/w}$), which is linked to several pharmacological property models such as toxicity, metabolism, distribution, and absorption, determines a compound's effective solubility in a non-aqueous medium (Alam & Khan, 2018). As a result, the ligand with lower $P_{o/w}$ value will partition itself more in the water phase, and vice versa. A key element of Lipinski's Ro5 (Lipinski et al., 2001) recommendations, which forecast a novel synthetic

compound's potential for use as oral drug, is lipophilicity. For optimal intestinal and oral absorption, an oral drug should, as opined by Lipinski's Rule of 5 (Ro5), have a LogP value <5. Optimally, this value should be between 1.35 and 1.8. Five publicly available prediction models are made available by SwissADME to predict lipophlicity: iLOGP, XLOGP3, WLOGP, MLOGP, and SILICOS-IT. The consensus (C) log $P_{o/w}$ is the average of the values predicted by these five proposed models. Findings from this work showed that all log $P_{o/w}$ values of the ligands were less than 5 based on Lipinski's Ro5, with isorhamnetin being the best (with a Log*P* of 1.66) (Table 2). Similarly, the lipophilicity at physiological pH 7.4 (LogD7.4) in which a compound with a value from 1-3 is deemed good according to this model, showed that the LogD values for glibencalmide, sitagliptin, liquiritin, and isorhamnetin were predicted to fall within the range values. From the other physicochemical properties predicted, the unsaturation and flexibility of the chosen ligands or compounds are assessed using the fraction of Csp3, which should be between 0.25 and 1, and the number of RB, which shouldn't be greater than 9 respectively. The TPSA of a molecule is a popular medicinal chemistry property used to optimize a drug's capacity to penetrate cells. Pajouhesh & Lenz (2005) have reported that compounds with a TPSA greater than 140Å^2 frequently exhibit restricted membrane penetration and are categorized as poorly absorbed. Also, according to Lipinski Ro5, a drug-like compound should not have MW> 500mg/mol, No of HBA>10, and No of HBD>5. From this result (Table 2), only acarbose has MW> 500mg/mol, No of HBA>10, and No of HBD>5. The lower the MW, the better because diffusion is directly affected. Also, repaglinide and liquiritin have their respective TPSA>140Å^2. Thus, since the predicted physicochemical properties of the four phenolics identified from the ethylacetate fraction of *A. conyzoides* were within the acceptable limit of a drug-like molecule for all the predicted physicochemical properties, they could be a potential oral drug candidate. The parameters such as HIA or GIA, MDCK permeability, human Caco-2 cell permeability, percentage human oral bioavailability (20% and 30%), and skin permeability coefficient (K_p), were used to assess the absorption and distribution potential of these ligands (Table 3). Oral bioavailability is undoubtedly one of the most important PK properties for any drug administered orally, as it measures the extent of drug's delivery to the systemic circulation. From the results of absorption parameters predicted (Table 3), the HIA (predicted using ADMETLab 2.0) and GIA (predicted using SwissADME) followed the same trend except for glibenclamide. With the exception of isorhamnetin, other phenolics showed low GIA/HIA, just like acarbose. A substance is said to have a good Caco-2 permeability if it has a predicted value >-5.15 log cm/s. As shown in the result (Table 3), isorhamnetin has the Caco-2 >-5.15 log cm/s as rosiglitazone, sitagliptin, and repaglinide. The Caco-2 cell has been intensively employed as a stand-in for the human intestinal epithelium in drug permeability tests carried out *in-vivo* due to their functional and physical similarities, and has consequently become an important indicator for a viable drug candidate. Conversely, the MDCK cells were developed as a model for *in-vitro* permeability screening. Here all the ligands investigated have a high passive MDCK permeability of P*app* >20x10^{-6} cm/s, except isorhamnetin, which has a moderate permeability. Syringin is the least skin permeant, followed by liquiritin, while repaglinide is the highest. More so, all the ligands were predicted to have at least 20% oral bioavailability, with the exception of acarbose. However, the four identified phenolics were predicted to have less than 30% oral bioavailability as acarbose. This poor oral bioavailability of acarbose could be attributed to its very high water solubility and very low (negative) lipophilicity, which influence the bioavailability of a compound (Ottaviani et al., 2010). By and large, drug passes across a variety of membrane barriers (such as the target cell, hepatocyte membrane, gastrointestinal epithelial cells, blood capillary wall, glomerulus, and restrictive organ barriers like the blood-brain barrier) during its absorption by the body (Alam & Khan, 2018).

Once an oral drug is absorbed, it is rapidly disseminated via the blood to numerous target organs, tissues, and cells where its actions are perceived. Until they are freed from plasma proteins, drug molecules attached to them have no pharmacological impact. This distribution process is influenced by different parameters such as PPB, BBB, VD, and Fu, as shown in the result of this study (Table 4). A drug's PD behaviour is greatly influenced by its binding to proteins in plasma, as PPB is one of the important routes of drug absorption and distribution. Due to the fact that as a drug binds to serum proteins, its free concentration is at risk, the PPB has a direct impact on oral bioavailability. As indicated in the result, most of the ligands have a PPB value < 90%, except for glibenclamide, rosiglitazone, repaglinide, and isorhamnetin. Juxtaposing this predicted PPB value with that of the Fu (fraction unbound) in plasmas, there is somewhat inverse relationship (Table 4). A ligand is said to have an ideal PPB if it has a predicted value < 90%, and drugs with good protein- binding may have a low therapeutic index. Most drugs in plasma will exist in equimolar amount between either an unbound state or bound to serum proteins. The degree to which a medicine binds blood proteins may have an impact on its effectiveness since a drug that is more tightly bound is less able to disperse or pass through cellular membranes. Acarbose, metformin, and syringin had very low PPB and high Fu values, respectively, relative to other ligands. If a ligand's estimated VD values fall between 0.04 and 20 L/kg, it is deemed to have a proper VD. In this study, all the ligands predicted can be said to have good VD since their respective values fall within the recommended range, with isorhamnetin having the highest VD value (0.65 L/kg) among other identified phenolics. As concerning the blood-brain barrier (BBB), drugs that work in the central nervous system (CNS) need to traverse the BBB to interact with their molecular target. However, for drugs with a peripheral target, no BBB permeation might be required so as not to cause CNS toxicity. Here, both ADMETLab 2.0 and SwissADME tools were used to predict the BBB values. Except for

acarbose and sitagliptin, all other ligands were predicted to be non-BBB-permeant using both tools (Table 4). A drug that is non-blood-brain permeant decreases the likelihood of causing harmful effects in the brain and blood stream when metabolized. Thus, the four identified compounds could be peripheral oral drug candidates, and may not cause harmful effects in the brain and blood stream because they cannot cross BBB.

More so, the drug concentration in the body may build up due to sluggish elimination process. It lowers the quantity of the medicine at the target site, which has an impact on its medicinal efficacy. The two main factors that influence drug elimination are clearance (CL) and drug half-life ($T^1/_2$). Since a drug's half-life is a composite concept involving both CL and VD, having precise estimations of these two qualities is arguably more suitable. The predicted CL result interpretation was as follows: >15 ml/min/kg-high clearance; 5–15 ml/min/kg-moderate clearance; <5 ml/min/kg-low clearance. Rosiglitazone has a predicted moderate CL tendency from the system, as well as isorhamnetin and sitagliptin, whereas acarbose had the lowest, followed by glibenclamide (Table 4).

Predicting if the ligands under study will likely be substrates or inhibitors of significant PK-related proteins such as permeability glycoprotein (P-gp) and cytochrome P450 (CYP) is therefore essential. The ligands under examination were assessed to identify whether the chemical may act as a substrate and/or an inhibitor of P-gp and CYPs in order to gain a better understanding of the mechanisms of drug distribution, efficacy, and toxicity. From the findings of the prediction of the inhibition potential, most of the ligands were found to follow a similar pattern when the outputs from the three web servers were juxtaposed together (Table 5), except for rosiglitazone and repaglinide. For instance, glibenclamide was predicted to be an inhibitor of all the CYP isozymes except for 2C19 (from ADMETLab 2.0 only). Interestingly, metformin, acarbose, furocoumarinic acid, liquiritin, and syringin (except for 2C9 in SuperCYPsPred) were predicted to be non-inhibitors of all the CYP isozymes. However, isorhamnetin was found to be an inhibitor of 1A2 and 3A4, and a non-inhibitor of 2C9, 2C19, and 2D6, from the three tools. Inhibition of these isozymes is probably one major causes of PKs-related DDIs, culminating to toxic or other unwanted adverse effects due to the slower elimination, and hence buildup of the drug or its metabolites (Kirchmair et al., 2015; Huang et al., 2008). With respect to the substrate effect of the ligands (Table 6), glibenclamide, for instance, was found to be a substrate for all the isoforms except for 2D6 and 1A2 (only in ADMETLab 2.0). Metformin showed variations in 2C19 and 2D6, while acarbose exhibited only variation in 2C19 (as substrate) and as non-substrate for other isoforms. More so, furocoumarinic acid was predicted to be non-substrates but for 2C9 (in ADMETLab 2.0), while liquiritin was found to be substrates for only 2C9 and 2D6 in ADMETLab 2.0. Similarly, syringin was found to be a non-substrate for all the isoforms using both servers. However, isorhamnetin was predicted not to be metabolized by 1A2 and 3A4 using SuperCYPsPred, and 3A4 only for ADMETLab 2.0. So, drug metabolism is crucial for both medication bioavailability and drug-drug interactions (DDIs) (Palleria et al., 2013). In comparison with some published works, a report by Do et al. (2014) demonstrated that metformin does not undergo significant metabolism with the common CYP450 isozymes but CYP1A1 and 1B1, which is consistent with this result except for 2D6, that was predicted to metabolize the drug. Similarly, Chen et al. (2014) reported that glibenclamide is metabolized by CYP2C9, 2C19, and 3A4 but can also inhibit 2C9 and 3A4. This was predicted by the three tools employed in this study. Studies have equally shown that repaglinide metabolizes CYP3A4 and 2C19 and also inhibits CYP3A4 (Bidstrup et al., 2003), as presented in this study. The most prevalent human hepatic CYP isoform is CYP3A4, which is involved in the metabolism of virtually 50% of clinically approved medications (Kato, 2019). An unwanted inhibition in CYP3A4 by co-administered drugs can result in clinically harmful DDIs. The expression of the CYP1A2 gene has been shown to be upregulated in diabetic patients (Chen et al., 2018; Matzke et al., 2000) and diabetic-induced animal models (Lee et al., 2009; Kim et al., 2005). The CYP2C families metabolized about 20% of pharmaceuticals available on the market, with the CYP2C9 isoform accounting for 60% of these metabolites (Hirota et al., 2013). This isoform partook in many clinically germane drug interactions, and is responsible for the metabolism of 15% of the clinically approved drugs (Hirota et al., 2013; Martignoni et al., 2006). The genetic variation of this enzyme could impact a number of clinically significant medications with a limited therapeutic range. Conversely, CYP2D6 makes up only 2-4% of all hepatic CYPs, although it is in charge of roughly 30% of all marketed medications (Kato, 2019; Matzke et al., 2000). By and large, when two or more drugs is metabolized by the same CYP, it is feasible that its metabolism could be blocked because of the competition between the drugs for the same binding site (Chen et al., 2018). So it will be essential to reduce the dosage of the drugs to minimize the adverse side effects through substrate-substrate interactions. In the same way, co-administering drugs that have inhibitory effects and are substrates of one specific CYP should be counterbalanced by decreasing the dosage because they stay longer in the organism than in monotherapy. So, not adjusting the dosage upscale the risk of even more side effects. Unlike the effect of the ligands on the CYPs, the result of their effect on P-gp did not appreciably follow a consistent pattern (Table 7). SwissADME only showed results for whether the ligand is substrate ("Yes") or non-substrate ("No"), while ADMETLab 2.0 showed both inhibitory and substrate effects of the ligands. Comparing the results from the two tools, it was only acarbose that was predicted to be substrate, while rosiglitazone, furocoumarinic acid, isorhamnetin, and syringin were non-substrates to P-gp using both tools. The rest of the ligands respectively, showed opposite effects (Table 7). As for the inhibitory effects, all the ligands were found

to be non-inhibitors ("0") of P-gp but glibenclamide. P-glycoprotein moderates the cellular absorption of drugs from blood stream into the brain and from the intestinal lumen into epithelial cells, alongside, exporting drugs for hepatic and renal excretion, thereby playing a crucial role in drug absorption and disposal (Liang et al., 2015; Lin & Yamazaki, 2003), and defense of the CNS from xenobiotics (Szakács et al., 2008). Comparably, P-gp protein, a family of the ABC (ATP-binding cassette) transporters, plays a crucial role in determining how much active efflux occurs across biological membranes. It is the most researched transporter in diabetic conditions and the main class of multi-drug-resistant transporters (MDRs) (Liu & Liu, 2014; Kobori et al., 2013). Thus, all the phenolics identified from the ethylacetate fraction of *A. conyzoides* methanol leaf extract will have no interference with the P-gp primary functions, as no inhibitory action was predicted. The experimental *in-vitro* α-amylase and α-glucosidase inhibition assays were carried out using the isorhamnetin-containing sample fraction. The PKs result analyses showed that isorhamnetin had relatively best *in-silico* ADME characteristics as can be inferred from its high oral bioavailability, water solubility, and lipophilicity as well as high GIA or HIA (Tables 2 and 3). From the *in-vitro* α-amylase inhibition assay result, the percentage inhibition of both the acarbose (standard) and isorhamnetin-containing sample were concentration-dependent. Reaching a concentration of 100µg/mL or higher, the enzyme tends to be saturated, resulting in approximately equal inhibition. However, the IC_{50} value of acarbose (50.33µg/ml) was slightly better than that of isorhamnetin-containing sample (54.42µg/ml) (Table 8). This could be because the isorhamnetin sample was still not a pure compound. Nonetheless, a Two-sample t-test comparison indicated that there is no statistically significant difference between the mean % inhibition of acarbose and isorhamnetin-containing sample, with t(df) = 0.42 (9), and a P-value = 0.340 ($P<0.05$). Similarly, the results of *in-vitro* α-glucosidase inhibition evaluation followed a similar pattern as that of α-amylase, but with higher inhibition values. However, the isorhamnetin IC_{50} value (15.97µg/ml) was lower and better than that of acarbose (18.11µg/ml) (Table 9). So, isorhamnetin-containing sample exhibited better inhibitory activity towards α-glucosidase than α-amylase. Despite the differences in inhibitors concentrations at 50% enzyme inhibition, a Two-Sample t-Test comparison indicated that there is no statistically significant difference between the mean % inhibition of acarbose and isorhamnetin-containing sample with t(df)= -0.26(10) and P-value = 0.601 ($P<0.05$). Through reduction of the extent at which carbohydrates are metabolized into simple sugars, inhibitors of *β*-glucosidase lower postprandial glucose spikes and help regulate blood glucose concentrations, which is an excellent method to manage diabetes (Ghani, 2015), as demonstrated in this present study. Generally, isorhamnetin is flavonol of the flavonoid class. Despite myriad of research reports about its biological and pharmacological activities (Liqing et al., 2016; Zhao et al., 2016; Antunes-Ricardo et al., 2015; Lee et al., 2005), isorhamnetin, to utmost of our knowledge, has not been documented to be present in *A. conyzoides*. Isorhamnetin could play role as a powerful antioxidant that protects cells from deleterious toxins, just like other flavonols. This molecule has enormous biological efficacies, including anticancer effects (Zhao et al., 2016), cardiovascular protection (Liqing et al., 2016), anti-inflammatory effects (Antunes-Ricardo et al., 2015), hepatoprotective action (Dong et al., 2014), and antidiabetic effect (Lee et al., 2009).

Conclusion

The *in-silico* pharmacokinetics studies indicated that the physicochemical properties of the four phenolics were within the acceptable limit of drug-like molecules with good water solubility, LogP, and LogD7.4. Apart from isorhamnetin, the other three phenolics exhibited low intestinal absorption. However, all the ligands were predicted to have at least 20% oral bioavailability, with the exception of acarbose. Although all the ligands were predicted to have good VD since their respective values fall within the recommended range, with isorhamnetin having the highest value (0.65 L/kg) among other identified phenolics. Also, all the identified phenolics were predicted to be non-inhibitors of the five main CYP450 isozymes, but 1A2 and 3A4 were inhibited by isorhamnetin. Similarly, they are mostly not metabolized by the isozymes, but 2C9, 2C19, and 2D6 were predicted to be metabolized by isorhamnetin. I*n-vitro* α-amylase and α-glucosidase inhibition assays indicated that the % inhibition by both the acarbose (standard) and isorhamnetin-containing sample were concentration dependent. However, the inhibitory effects were more on α-glucosidase (IC_{50} of 18.11 and 15.97 µg/ml for acarbose and isorhamnetin, respectively) than on α-amylase (IC_{50} of 50.33 and 54.42 µg/ml). Thus, isorhamnetin isolated from this plant could serve as a good alternative oral antidiabetic drug.

Significant of the study: This research has displayed that the phenolics (furocoumarinic acid, isorhamnetin, liquiritin and syringin) from ethylacetate fraction of *A. conyzoides* have relatively good pharmacokinetics within the acceptable limit of drug-like molecules, and most of them were predicted as non-inhibitors of common CYP450 isozymes as well as P-glycoproteins.

List of abbreviations

AC	Absorbance of control
ADME	Absorption, distribution, metabolism, and excretion
AS	Absorbance of sample

BBB	Blood brain barrier
Caco-2	Human colon adenocarcinoma cell lines
CADD	Computer-aided drug design
CL	Clearance
CVDs	Cardiovascular diseases
CYP	Cytochrome P450
DM	Diabetes mellitus
F	Human oral bioavailability
Fu	Fraction unbound in plasm
GIA	Gastrointestinal absorption
HBD	Hydrogen bond donor
HDA	Hydrogen bond acceptor
HIA	Human intestinal absorption
I	Inhibitor
K_p	Skin permeability coefficient
LogP	Partition coefficient or lipophilicity
MDCK	Madin−Darby Canine Kidney cells
MR	Molecular refractivity
MW	Molecular weight
NCBI	National Center for Biotechnology Information
P-gp	Permeability glycoprotein
PK	Pharmacokinetic
PPB	Plasma protein binding
RB	Rotatable bond
S	Substrate
SBDD	Structure-based drug design
SS	Skin sensitivity
$T^1/_2$	Half-life
TPSA	Topological polar surface area
VD	Volume distribution

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Author contributions

PC Ozioko conceptualized and designed the research idea under the supervisions of A Ibrahim. PC Ozioko and A Ibrahim analyzed the results. The manuscript was written by PC Ozioko and SA Abdullahi under the supervision of. A Ibrahim. All the authors approve the manuscript.

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