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In Silico Toxicity Prediction of Ethanol Extract of *Cola rostrata* (K. Schum.) Epicarp

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Abstract

The ethanol extract of the epicarp of *Cola rostrata* fruit has been reported to possess notable pharmacological properties, including anticancer, anti-inflammatory and antidiabetic effects; however, its toxicological profile remains understudied. This study evaluated the metabolism, excretion and toxicity properties of Gas Chromatography-Mass Spectroscopy-identified phytochemicals from *C. rostrata* epicarp. *In silico* analysis and molecular docking of components were carried out using the ADMETlab2.0 platform and Autodock4 tools. Visualization of molecular binding interactions was done using Discovery Studio-2020. Ten of the 48 compounds in the extract, including 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene, Anthiaergostan-5,7,9-trien-14.alpha.,15.alpha.-diol and 2-Hydroxychalcone, were predicted to have high probability of inducing liver injury, oxidative stress and inhibiting cytochrome-P450 enzymes. Molecular docking revealed that 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene binds strongly to NADH dehydrogenase 1 (-7.78 kcal/mol) and CYP2C19 (-9.93 kcal/mol), with the compound interacting with Thr301, Leu361 and Leu366 at the active site of CYP2C19. 2-Hydroxychalcone binds strongly to CYP2C19 (-8.07 kcal/mol) and to Na^+/K^+-ATPase (-7.49 kcal/mol), while, Anthiaergostan-5,7,9-trien-14.alpha.,15.alpha.-diol binds strongly to CYP2C19 (-9.56 kcal/mol) and CYP1A2 (-8.71 kcal/mol). The extract showed strong potential to induce toxic outcomes. The abundance of antioxidant phytosterols in the extract may counterbalance the potential toxicity. While *C. rostrata* holds therapeutic potential, molecular interactions of its phytochemicals highlight risks of toxicity.

Keywords: *Cola rostrata*; Phytochemicals; Metabolism; Toxicity profile; GC-MS; Molecular docking.

INTRODUCTION

A large majority of people in developing areas of the world depend on natural products for the treatment and management of various diseases and illnesses (Ekor, 2014; Mintah *et al*., 2022). The current global hike in the prices of clinically used drugs, chemotherapeutic drugs in particular, is making more people choose plant-based remedies, owing to their affordability, accessibility, and availability (Shin *et al*., 2022; Serra-Burriel *et al*., 2023; Vasques *et al*., 2023). Plants have proven to be a viable source of bioactive compounds that have been developed into drugs for treating several disease conditions (Newman & Cragg, 2020). The increase in the usage of plant remedies for healthcare has come with more research attention and warnings on the safety of these remedies and their potential to induce adverse reactions (Okaiyeto & Oguntibeju, 2021: Başaran *et al*., 2022). The adverse effects reported for some plant extracts include, liver damage, injuries to tissues of the gastrointestinal tract, kidney failure, cerebral haemorrhage etc. (Posadzki *et al*., 2013; Frenzel & Teschke, 2016).

Extracts of Cola species are used in traditional medicine for the treatment of various diseases and ailments, with some of them used in the management of diabetes and cancer (Ekalu & Habila, 2020; Ngoka *et al*, 2021). *Cola rostrata* is a member of the genus that has limited information in the literature on the toxicity of its extracts. The extract of the root bark of *C. rostrata*, which contains flavonoids, phenols, steroids, tannins saponins, triterpenoids and alkaloids was found to be safe after the acute toxicity of the extract was assessed in mice (Odion *et al*., 2013). Dongmo *et al* (2019) found weak cytotoxic activity in the prenylated derivative of compounds isolated from the root of *C. rostrata* against human cervix carcinoma KB-3-1 cells. Ajayi *et al* (2022, 2023) determined the components of the epicarp extract of *C. rostrata* and the protein targets of the drug-like compounds in it; they also found the extract to be significantly cytotoxic against MRC5-SV2 and HeLa cells with potential to be effective against diabetes, pain

and inflammatory diseases. The evaluation of absorption, distribution, metabolism, excretion and toxicity (ADMET) profile of the components of the crude extract of *C. rostrata* epicarp is yet to be done.

Generally, the toxicity of herbal preparations is a function of the contributions of each of the molecules in the extracts. The interactions among extract components could result in the elimination of the effect of some potentially harmful components or accentuation of the toxic effect of the few potentially harmful components. Reports show that in some cases, the direct interactions of extract components could be synergistic, antagonistic or additive in nature to bring about the observed pharmacological response in living systems, while in some other cases, there is indirect interaction, products of the metabolism of the constituent of the extracts may interact with one another to produce the observed effect (Matotoka & Masoko, 2018; Uduwana *et al*., 2023).

To assess the safety and mechanisms of pharmacological activities of the crude extract of *C. rostrata* epicarp as a remedy for potential use in traditional medicine against diabetes, inflammatory diseases, pain and cancer, this study evaluated the metabolism, excretion, toxicological properties, mode of action, and binding affinities of the compounds present in ethanol extract of the plant using *In silico* analyses and molecular docking. Molecular docking of most toxic components of the extract was carried out against selected proteins in the pathways perturbed by the extract fractions.

MATERIALS AND METHODS

Extract Preparation, Fractionation and Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The collection of plant materials, extract preparation, and GC-MS analysis of the extract have been previously reported by Ajayi *et al* (2022). Briefly, the epicarp of fresh ripe *C. rostrata* fruit was collected, cut into small pieces, air-dried at room temperature under shade for four months, and pulverized with an electric blender. The pulverized material was soaked in absolute ethanol for six days with frequent rigorous shaking of the extraction vessel to disperse phytochemicals. The crude extract was obtained by filtration, followed by concentration under pressure, and then air-drying. The air-dried crude extract was fractionated via liquid-liquid partitioning with 70% methanol solution and n-hexane to obtain a polar fraction and a non-polar fraction. The constituents of the fractions were identified via GC-MS, the identified compounds were reported by Ajayi *et al* (2022).

In silico Metabolism, Excretion and Toxicity Analyses of the Components of Extract Fractions

The simplified molecular input line entry system (SMILES) notation for each compound present in the two extract fractions was obtained from PubChem. The SMILES were submitted to ADMETlab 2.0 online platform for systematic evaluation of the metabolism, excretion and toxicity properties of each constituent of the extract fractions. For each constituent of the extract fractions, the probabilities of having specific properties that are known to define the toxicity of known drug compounds were scored; these properties include, permeation of the blood-brain barrier, clearance (excretion), carcinogenicity, inhibition of members of Cytochrome families, induction of liver injury, and inhibition of Tox21 pathway proteins. The probabilities of the constituents binding to these proteins and inhibiting their actions (or inducing toxic responses) were reported as follows: probability 0.0–0.1 as no binding, 0.1–0.3 as very low inhibition potential, 0.3–0.5 as low inhibition potential, 0.5–0.7 as medium inhibition potential, 0.7–0.9 high inhibition potential and 0.9–1.0 as having very high inhibition potential; with the very high inhibition potential corresponding to the most profound toxic effect.

Molecular Docking

Based on the toxicity potential of the extract constituents, the most toxic components were identified via cross-tabulation of the scores of their toxicity properties. The identified compounds were docked against CYP1A2, CYP2C19, Na^+/K^+–ATPase, and NADH dehydrogenase 1. Cytochrome P450 members are important in the metabolism of xenobiotics, their impairment have been reported to have profound toxicological implications in human cells (Ogu & Maxa, 2000; Guengerich, 2022). Na^+/K^+–ATPase and NADH dehydrogenase 1 are important for the maintenance of cellular redox balance and mitochondrial membrane potential (Pivovarov *et al*., 2019; Herrmann & Riemer, 2021).

Preparation of Protein Molecules

The crystal structures of human CYP1A2 (PDB ID: 2HI4), CYP2C19 (PDB ID: 4GGS), Na^+/K_+–ATPase (PDB ID: 7E1Z), and NADH dehydrogenase 1 (PDB ID: 5XTD) were downloaded from Protein Data Bank (www.rcsb.org). Using AutoDock4 molecular docking software, the protein subunits (polypeptide chains) of CYP2C19 and Na^+/K^+–ATPase were separated and treated individually; CYP1A2 comprises a single polypeptide chain. NADH dehydrogenase 1 comprises 44 subunits, the chains were divided into three clusters based on proximity to one another; Cluster 1 contains 8 polypeptide chains: A, F, K, L, M, N, O and T; Cluster 2 contains 16 polypeptide chains: B, C, E, G, H, I, J, j, m, P, Q, s, S, U, w and W; while Cluster 3 contains 20 polypeptide chains: a, b, c, d, e, f, g, h, i, k, l, n, o, p, r, v, V, X,Y and Z. Water molecules, other heteroatoms, and non-standard amino acid molecules were deleted from the polypeptide chains. Non-polar hydrogen atoms and Kollman charges were added to the chains. The

polypeptide chains were checked for missing atoms, and repairs of missing atoms were done. The atoms of the polypeptide chains were formatted as AutoDock4 molecules, and the chains were saved in pdbqt format.

Preparation of Ligand Molecules

Using the "Draw Structure" provision on PubChem platform, the SMILES notation of three components of the fractions were used to obtain their MDL Molfile which were then converted to Mol2 file with Discovery Studio 2021 software. The structures were converted to their pdbqt format on AutoDock4 after hydrogen atoms had been added to each compound and the Gasteiger charges computed.

Docking Experiments

Blind docking was done with the grid box coordinates covering the entire atoms of the polypeptide chains, but in few cases, due to the size of the polypeptide chains some atoms were not covered by the grid. The Genetic Algorithm (GA) parameters include, Number of GA runs (30), and Population size (300). For each docking experiment, the best pose i.e. the one with the highest binding energy, and the binding interactions between the ligands and the polypeptide chains were visualized on Discovery Studio 2021 software.

RESULTS AND DISCUSSION

Results

Chemical constituents of polar and non-polar fractions of *C. rostrata* epicarp were identified using GC-MS analysis. The inhibitory effects of these components on CYP450 family enzymes were evaluated as presented.

GC-MS Identification of Constituents

The constituents of the polar and non-polar fractions of the epicarp of *C. rostrata* identified via GC-MS and their proportion (Area %) in the fractions are presented in Table 1. A total of 51 compounds were identified in the fractions, 29 in the non-polar fraction and 22 in the polar fraction, with Heptadecanoic acid methyl ester, n-Heptadecanoic acid and Stigmasterol co-partitioning into both fractions.

Inhibitory Effects of Components of the Extract Fractions on CYP450 Family Members

The detoxification of drug compounds in humans is chiefly done by members of the Cytochrome P450 (CYP) families. The potential of the components of the polar and non-polar fractions of *C. rostrata* epicarp extract to bind to and inhibit members of CYP family is presented in Table 2. A total of 10 compounds, five from each extract fraction, have very strong potential (probability between 0.9 and 1.0) to inhibit CYP1A2, these 10 compounds are (i) 3-Cyclopentylpropionic acid, ethyl ester, (ii) cis-9-Tetradecenoic acid, propyl ester, (iii) Z,Z-10,12-Hexadecadien-1-ol acetate, (iv) 7,10-Octadecadienoic acid, methyl ester, and (v) Z-8-Methyl-9-tetradecen-1-ol formate from the non-polar fraction; and (vi) 7-Pentadecyne, (vii) Silicic acid, diethyl bis(trimethylsilyl) ester, (viii) 4-Methoxy-6-methyl-5-nitroisobenzofuran-1,3-dione, (ix) 2-Hydroxychalcone, and (x) Corynan-16-carboxylic acid, 16,17-didehydro-9,17-dimethoxy-, methyl ester, (16E)- from the polar fraction. These last three compounds listed above (viii, ix and x) also have strong potential to inhibit CYP2C19. 2-Hydroxychalcone, compound ix in the list above, could also inhibit CYP2C9 and CYP3A4 strongly, while Anthiaergostan-5,7,9-trien-14.alpha.,15.alpha.-diol, which is also present in the polar fraction of the extract, has very strong potential to inhibit CYP2D6.

Table 1. Components of the fractions of ethanol extract of *Cola rostrata* epicarp

SN	Non-polar Fraction	Area%	Polar Fraction	Area%
1	3-Cyclopentylpropionic acid, ethyl ester	0.60	Hexadecanoic acid, methyl ester	1.48
2	Azelaic acid, monoethyl ester	1.20	n-Hexadecanoic acid	8.57
3	Tetradecanoic acid	0.67	Bicyclo[2.2.2]octane, 2-methyl-	1.55
4	Pentadecanoic acid	1.19	2-Myristynoyl-glycinamide	1.29
5	Hexadecanoic acid, methyl ester	0.80	Oleic Acid	2.89
6	n-Hexadecanoic acid	27.45	Methyl 9,12-heptadecadienoate	1.59
7	Hexadecanoic acid, ethyl ester	6.59	7-Pentadecyne	1.24
8	cis-9-Tetradecenoic acid, propyl ester	0.80	2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4a.alpha.,7.beta.,8a.beta.)-	6.93
9	Heptadecanoic acid	1.69	(3R,4aS,8aS)-8a-Methyl-5-methylene-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene	4.96
10	Z,Z-10,12-Hexadecadien-1-ol acetate	0.66	13-Tetradece-11-yn-1-ol	5.34
11	Octadecanoic acid	7.09	Silicic acid, diethyl bis(trimethylsilyl) ester	2.28
12	Octadecanoic acid, ethyl ester	2.09	2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3.alpha.,5a.alpha.,9.alpha.,9a.alpha.)]-	1.56
13	9,12-Octadecadienoic acid (Z,Z)-	0.76	Cyclododecanol, 1-aminomethyl-	1.38
14	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	1.68	2-[1-(3,4-Dimethyl-phenyl)-1H-tetrazol-5-ylsulfanyl]-N-phenethyl-acetamide	3.66
15	7,10-Octadecadienoic acid, methyl ester	0.59	2-Hydroxychalcone	1.76
16	Z-8-Methyl-9-tetradecen-1-ol formate	0.86	4-Methoxy-6-methyl-5-nitroisobenzofuran-1,3-dione	2.55
17	9,12-Octadecadienoic acid (Z,Z)-	5.32	Corynan-16-carboxylic acid, 16,17-didehydro-9,17-dimethoxy-, methyl ester, (16E)-	13.33
18	Ethyl 9.cis.,11.trans.-octadecadienoate	12.09	Anthiaergostan-5,7,9-trien-14.alpha.,15.alpha.-diol	8.21
19	Naphthalene, decahydro-2-methyl-	2.28	Methanesulfonic acid, 17-cyano-10,13-dimethylhexadecahydrocyclopenta[a]phenanthren-17-yl ester	13.68
20	2-Dodecen-1-yl(-)succinic anhydride	3.84	Stigmasterol	9.62
21	9-Tricosene, (Z)-	0.88	[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester	6.11
22	Stigmasta-4,22-diene	1.59		
23	β-Sitosterol	2.69		
24	Docosanoic acid	2.75		
25	3-(2-Methoxymethoxyethylidene)-2,2 dimethylbicyclo[2.2.1]heptane	0.79		
26	1-(4-Methoxyphenylazo)-2-phenoxynaphthalene	0.92		
27	γ-Sitosterol	6.13		
28	Stigmasterol	3.68		
29	Stigmasta-4,22-diene	2.33		

Table 2. Numbers of *Cola rostrata* extract components with various degrees of inhibitory binging to members of Cytochrome P450 protein family.

CYP Family	No Binding		Very low		Low		Medium		High		Very high	
	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P
CYP1A2	6	3	11	4	0	1	3	6	3	2	5	5
CYP2C19	7	3	9	9	5	1	5	3	2	2	0	3
CYP2C9	6	1	13	12	5	3	3	3	1	1	0	1
CYP2D6	17	13	8	4	1	2	2	0	0	1	0	1
CYP2A4	10	4	4	7	8	4	4	2	2	3	0	1

CYP: Cytochrome P450; **NP**: non-polar fraction; **P**: polar fraction. Probability of inhibition: No Binding (0–10%); Very low (10–30%); Low (30–50%); Medium (50–70%); High (70–90%) and Very high (90–100%).

Toxicity of the Components of *C. rostrata* Extract Fractions

The predicted toxic effects of the components of the polar and non-polar components of *C. rostrata* epicarp is mainly drug-induced liver injury, followed by hepatotoxicity and ability to block the hERG channels (Table 3). Two compounds, one in each of the fractions, have 90 % to 100 % likelihood of causing liver damage, and these compounds are 2-Hydroxychalcone and 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene, in the polar and non-polar fractions, respectively. Silicic acid, diethyl bis(trimethylsilyl) ester which is present in the polar fraction, also has 70 % to 90 % likelihood of causing injury to the liver. In the non-polar fraction, Ethyl 9.cis.,11.trans.-octadecadienoate and β-Sitosterol have high likelihood of blocking hERG channels. Hepatotoxicity could be caused by two compounds in the polar fraction: Methanesulfonic acid, 17-cyano-10,13-dimethylhexadecahydrocyclopenta[a]phenanthren-17-yl ester and [1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester. Other predicted negative effects of the components include the likelihood of 3-(2-Methoxymethoxyethylidene)-2,2 dimethylbicyclo[2.2.1]heptane and Corynan-16-carboxylic acid, 16,17-didehydro-9,17-dimethoxy-, methyl ester, (16E)-inducing carcinogenesis, and 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene with a very high potential to induce mutation.

Table 3. Numbers of *Cola rostrata* extract components with various degrees of end-point toxicities.

End-point Toxicities	Nontoxic		Very low		Low		Medium		High		Very high	
	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P
hERG Blockers	18	13	6	7	1	0	1	0	2	0	0	1
Hepatotoxic	20	9	7	8	1	2	0	0	0	2	0	0
DILI	17	11	6	3	2	2	2	2	0	2	1	1
Mutagenicity	27	17	0	1	0	0	0	2	0	1	1	0
ROAT	26	14	2	2	0	2	0	1	0	1	0	1
FDAMDD	18	9	1	6	4	1	4	1	1	1	0	3
Carcinogenicity	14	10	9	3	2	0	1	5	1	2	1	1

hERG: human Ether-a-go-go-Related Gene; DILI: drug-induced liver injury; ROAT: rat oral acute toxicity; FDAMDD: Federal Drug Administration maximum (recommended) daily dose; NP: non-polar extract fraction; and P: polar extract fraction. Probability of toxicity: Nontoxic (0–10%); Very low (10–30%); Low (30–50%); Medium (50–70%); High (70–90%) and Very high (90–100%).

The predicted binding (activatory or inhibitory) effect of the components of the extract fractions to proteins identified by the Tox21 program as significant predictors of toxicity is shown in Table 4. A total of 11 compounds in both extract fractions have from high to very high potential to bind to the nuclear receptor protein, Peroxisome Proliferator-Activated Receptor Gamma (NR-PPAR-Gamma). Out of these 11 compounds, eight are in the non-polar fraction, they are fatty acids and fatty acid derivatives, and they make up about 43% proportion by mass in the extract fraction. 2-Hydroxychalcone is the only non-fatty acid component of the polar extract fraction that could bind strongly to NR-PPAR-Gamma. From the two extract fractions, a total of 10 compounds could strongly induce the stress response pathway leading to the disruption of mitochondrial membrane potential (SR-MMP). 2-Hydroxychalcone, Anthiaergostan-5,7,9-trien-14.alpha.,15.alpha.-diol, Methanesulfonic acid, 17-cyano-10,13-dimethylhexadecahydrocyclopenta[a]phenanthren-17-yl ester, and Stigmasterol are compounds that could disrupt mitochondrial membrane potential in the polar fraction, and in the non-polar fraction of the extract, the same effect could be induced by 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene, Naphthalene, decahydro-2-methyl-, 2-Dodecen-1-yl(-)succinic anhydride, γ-Sitosterol, Stigmasterol, and Stigmasta-4,22-diene.

Table 4. Numbers of *Cola rostrata* extract components with inhibitory binding potential against TOX21 proteins.

	No binding		Very low		Low		Medium		High		Very high	
	NP	P	NP	P	NP	P	NP	P	NP	P	NP	P
NR-AR	14	17	4	0	8	3	1	0	1	1	0	0
NR-AR-LBD	28	20	0	0	0	0	0	1	0	0	0	0
NR-AhR	26	16	0	1	0	1	1	1	0	0	1	2
NR-Aromatase	18	12	6	4	1	2	3	1	0	1	0	1
NR-ER	3	7	12	8	12	4	0	1	0	0	1	1
NR-ER-LBD	19	12	1	3	3	1	1	2	2	2	2	1
NR-PPAR-Gamma	14	12	4	3	2	2	0	1	0	1	8	2
SR-ARE	11	10	10	4	5	5	1	1	0	0	1	1
SR-ATAD5	26	20	1	0	0	0	0	0	0	1	1	0
SR-HSE	8	9	11	4	3	5	3	1	1	1	2	1
SR-MMP	6	4	11	6	5	3	0	4	4	1	2	3
SR-P53	25	16	1	1	0	2	0	1	0	0	2	1

Estrogen Receptor-Ligand-Binding Domain (NR-ER-LBD Peroxisome Proliferator-Activated Receptor Gamma (NR-PPAR-Gamma) mitochondrial membrane potential (SR-MMP) tumor suppressor protein (SR-p53), Heat shock factor response element (SR-HSE), Antioxidant response element (SR-ARE), Estrogen receptor (NR-ER), Aryl hydrocarbon Receptor (NR-AhR) and ATPase family AAA domain-containing protein 5 (SR-ATAD5).

A total of seven compounds (mostly those with the gonane nucleus) have high to very high probability of binding to Estrogen Receptor-Ligand-Binding Domain (NR-ER-LBD) and activating series of reactions that result in endocrine disruption. In the non-polar fraction, γ-Sitosterol, Stigmasterol and Stigmasta-4,22-diene have strong potential to bind to the ligand-binding domain of estrogen receptor; in the polar fraction of the extract, the same potential is seen in 2-Hydroxychalcone, Anthiaergostan-5,7,9-trien-14.alpha.,15.alpha.-diol and Stigmasterol. Among all the compounds identified in the two extract fractions, 2-Hydroxychalcone is the most toxic component of the epicarp extract of *C. rostrata* since it could cause liver damage, disrupt mitochondrial membrane potential, modulate sugar metabolism through NR-PPAR-Gamma activation, and disrupt endocrine function through its effect on the estrogen receptor. In addition to 2-Hydroxychalcone's inhibitory effects on members of the Cytochrome P450 family, it could also bind strongly to and modulate the functions of tumor suppressor protein (SR-p53), Heat shock factor response element (SR-HSE), Antioxidant response element (SR-ARE), Estrogen receptor (NR-ER), Aryl hydrocarbon Receptor (NR-AhR) and ATPase family AAA domain-containing protein 5 (SR-ATAD5). Next to 2-Hydroxychalcone is 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene which also has strong potential to induce liver damage and mutation, and disrupt mitochondrial membrane potential. 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene could also predict to bind strongly to and modulate the functions of SR-ARE, NR-AhR, NR-ER, SR-ATD5, and members of the CYP450 family, while also disrupting mitochondrial membrane potential. Some of the compounds present in the extract that have been predicted to cause toxic outcomes are presented in Figure 1.

Analysis of Binding Energies

The docking experiment revealed that components of the fractions of *C. rostrata* epicarp extracts, 2-Hydroxychalcone, 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene, and Anthiaergostan-5,7,9-trien-14.alpha.,15.alpha.-diol, are potent inhibitors of CYP2C19 and CYP1A2; the three compounds showed strong binding affinity for the four subunits of CYP2C19, having average Free energy of -7.21, -9.65 and -8.62 kcal/mol, respectively. Table 4 shows the binding energies of selected components of the extract fractions with Na^+/K^+-ATPase, NADH Dehydrogenase and two members of the CYP450 family. Out of the three components of the extract used in the molecular docking experiment, 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene showed the strongest binding affinity for CYP2C19 and NADH Dehydrogenase with the compound binding strongly to each of the four subunits of CYP2C19. 2-Hydroxychalcone has the strongest affinity for Na^+/K^+-ATPase, binding to the B subunit with a free energy of –7.49 kcal/mol.

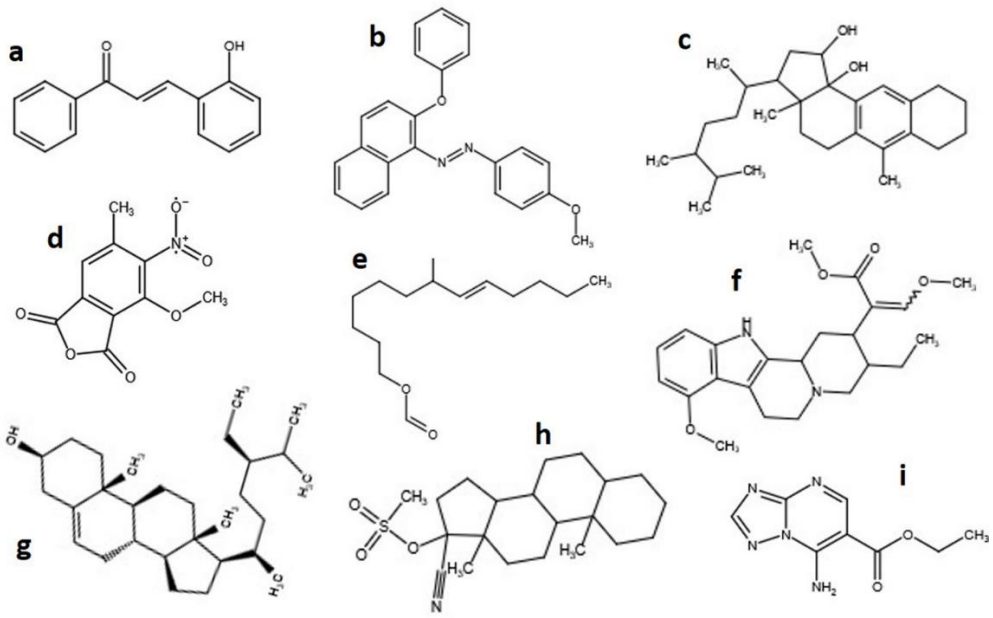

Figure 1. Some components of ethanol extract of *C. rostrata* epicarp predicted to have toxic effects. (a) 2-Hydroxychalcone, (b) 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene, (c) Anthiaergostan-5,7,9-trien-14.alpha.,15.alpha.-diol, (d) 4-Methoxy-6-methyl-5-nitroisobenzofuran-1,3-dione, (e) Z-8-Methyl-9-tetradecen-1-ol formate, (f) Corynan-16-carboxylic acid, 16,17-didehydro-9,17-dimethoxy-, methyl ester, (16E)-, (g) β-Sitosterol, (h) Methanesulfonic acid, 17-cyano-10,13-dimethylhexadecahydrocyclopenta[a]phenanthren-17-yl ester, and (i) [1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester.

Examination of the binding interactions showed that the most predominant interaction of 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene with the polypeptide chain D of CYP2C19 are Pi-Alkyl bonds with the interaction with conventional hydrogen bond having the shortest bond length of 2.93 Å (Figure 2 C). Other interactions with the polypeptide chain include van der Waals forces, and alkyl, Pi-Pi T-shaped, Pi-sulfur, Pi-sigma, and carbon-hydrogen bonds. The binding interaction of 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene with CYP2C19 shows that the compound binds to residues: Thr301, Leu361 and Leu366 at the active site of CYP2C19, with the free energy of -9.93 kcal/mol. Also, the binding interactions of 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene to NADH dehydrogenase 1 has Pi-alkyl bonds as the predominant interactions, nine bonds with residues on three subunits (r, i and V chains) in Cluster 3 of the NADH dehydrogenase 1 structure (Figure 2 A). The shortest bond distance (1.88 Å) and the strongest bond seen in the interactions is the conventional hydrogen bonding between lone pairs of electrons on the hydroxyl group of 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene and Glu V:134. Other bonds in the binding interaction include Pi-Pi stacked, Pi-Pi T-stacked and Pi-donor hydrogen bonds. This binding of 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene to Cluster 3 chains has the lowest free energy (-7.78 kcal/mol) of the three compounds docked with the three chain clusters of NADH dehydrogenase 1 protein. The interaction of 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene with NADH dehydrogenase (a critical enzyme in the mitochondrial electron transport chain that plays a central role in cellular energy production through oxidative phosphorylation) suggests the potential modulation of ATP production.

Table 5. Binding free energy (kcal/mol) of three components of *Cola rostrata* extract to selected cellular proteins.

Components of *Cola rostrata* epicarp extract	Na^+K^+-ATPase			NADH Dehydrogenase 1			CYP2C19				CYP1A2
	A	B	C	Cluster 1	Cluster 2	Cluster 3	A	B	C	D	
2-Hydroxychalcone	-5.41	-7.49	-4.97	-5.48	-6.82	-5.77	-8.07	-7.11	-6.89	-6.77	-7.31
1-(4-Methoxyphenylazo)-2-phenoxynaphthalene	-5.76	-5.58	-5.13	-6.86	-6.46	-7.78	-9.68	-9.52	-9.49	-9.93	-7.51
Anthiaergostan-5,7,9-trien-14.alpha.,15.alpha.-diol	-6.00	-5.17	-5.25	-6.54	-7.09	-7.17	-9.56	-8.16	-7.91	-8.83	-8.71

Cluster 1 contains polypeptide chains: A, F, K, L, M, N, O and T; **Cluster 2** contains polypeptide chains: B, C, E, G, H, I, J, j, m, P, Q, s, S, U, w and W; **Cluster 3** contains polypeptide chains: a, b, c, d, e, f, g, h, i, k, l, n, o, p, r, v, V, X,Y and Z. **A, B, C and D**: subunits of the indicated proteins.

For the binding interactions of 2-Hydroxychalcone, its binding to Chain B of Na^+/K^+-ATPase has the lowest free energy (-7.74 kcal/mol) of the dockings of the three compounds to the chains of Na^+/K^+-ATPase (Table 5). The binding interaction of Hydroxychalcone to Chain B involves two conventional hydrogen bonds linking Met B:202 with bond distances of 1.90 Å and 2.25 Å, and Pi bonds: Pi-alkyl, Pi-anion, Pi-sulfur and Pi-Pi T-shaped; the Pi-sulfur bond is also linking Met B:202 (Figure 2 D). The binding of Anthiaergostan-5,7,9-trien-14.alpha.,15.alpha.-diol to CYP1A2 produced the best free energy value (-8.71 kcal/mol) of the three compounds docked with the protein (Table 5). The binding of Anthiaergostan-5,7,9-trien-14.alpha.,15.alpha.-diol to CYP1A2 involves alkyl bonding (9 bonds) with hydrophobic aliphatic side chains of Leu and Ile residues; with the bond with the shortest distance (3.46 Å) being the alkyl bond formed with Cys 458 (Figure 2 B). Other bond interactions in the binding of Anthiaergostan-5,7,9-trien-14.alpha.,15.alpha.-diol to CYP1A include, Pi-sigma, amide-Pi stacked and Pi-donor hydrogen bonds.

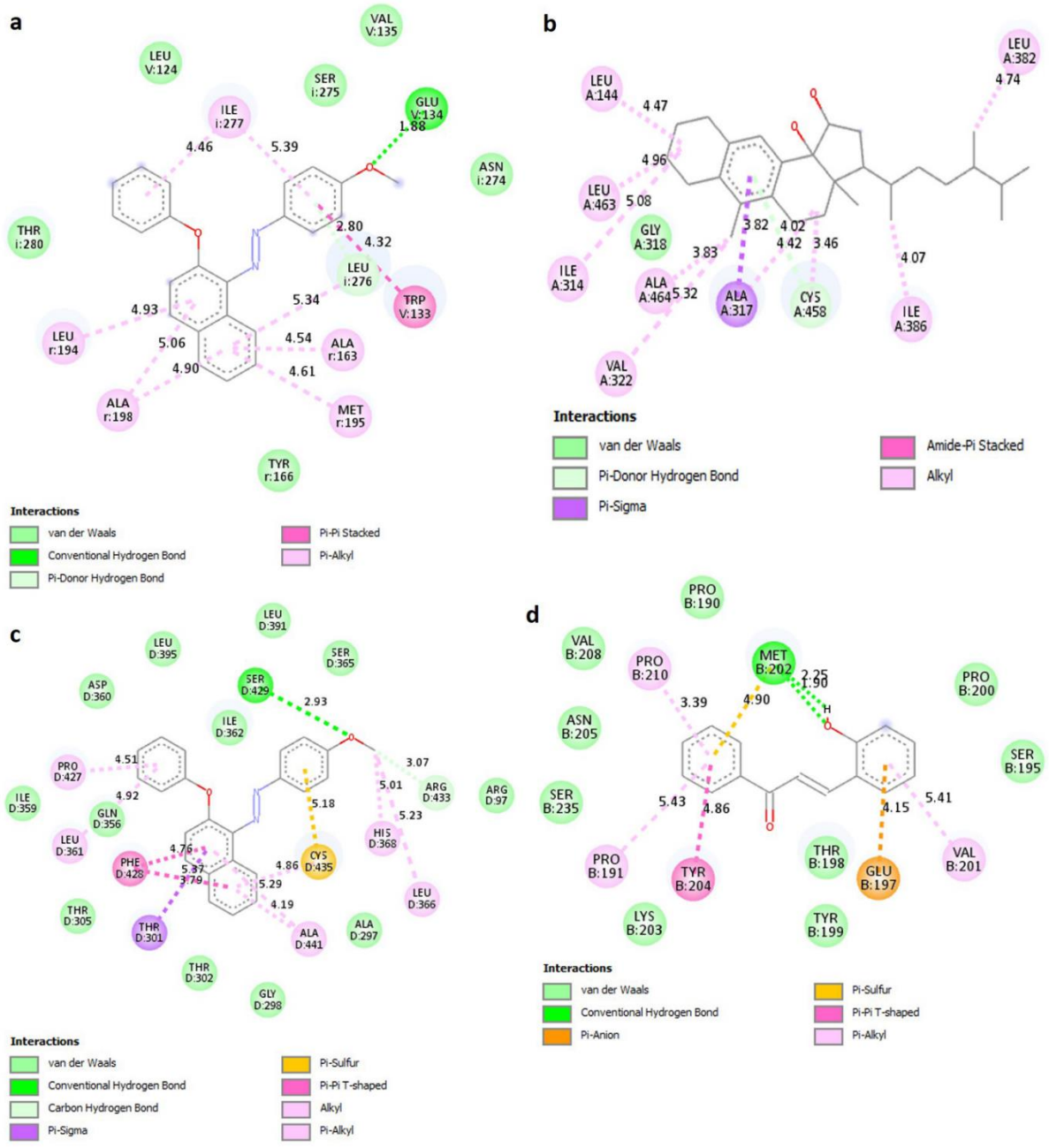

Figure 2. 2D Images of the molecular binding interactions of selected components of *Cola rostrata* extract with selected cellular proteins. (A) Binding interactions of 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene with amino acid residues in 20 subunits (a, b, c, d, e, f, g, h, i, k, l, n, o, p, r, v, V, X,Y and Z) of NADH dehydrogenase 1 (B) Binding interactions of Anthiaergostan-5,7,9-trien-14.alpha.,15.alpha.-diol to CYP1A (C) Binding interactions of 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene to Chain D of CYP2C19 (D) Binding interactions of 2-Hydroxychalcone to Chain B of Na^+/K^+-ATPase.

Discussion

The ethanol extract of the fruit epicarp of *Cola rostrata* has been found to possess strong anticancer activity with potential for usage against inflammation, pain and diabetes; the toxicity potential of this extract has not been evaluated. Adverse side effects have been identified as one of the barriers to increased usage of herbal preparations (Awodele *et al*., 2018; Vaezi *et al*., 2021). Though adverse reactions are associated with several herbal preparations, conventional orthodox drugs like carbamazepine, diclofenac and most antibiotics, including tetracycline, azithromycin andmoxifloxacin, have been identified as major causes of liver damage and adverse side effects (Chalasani *et al*., 2015; Mejia *et al*., 2020; Osuntokun *et al*., 2020). The high potential for adverse reactions with the use of herbal preparations is attributable to the huge diversity and the numerical enormity of phytochemicals that are consumed at the same time, and in most cases, in unstandardized quantities. With 10 components of the extract of *C. rostrata* epicarp possessing the ability to induce oxidative stress and initiate processes leading to loss of mitochondrial membrane potential and massive apoptotic cell death; red blood cells and brain cells which require a high level of oxygen could be sites for adverse reactions after the extract is consumed.

GC-MS and Toxicity analyses

The GC-MS analysis of components of ethanol extract of *C. rostrata* epicarp identified 48 different phytochemicals. This value represents the components of the extract that are already known, but possible novel components are not accounted for, and the possibility of having these unidentified components contributing significantly to toxic outcomes cannot be ascertained. The high potential of some of the identified components of the extract to cause liver damage relates well with the reported potential of the extract for the management of pain and inflammation as many non-steroidal anti-inflammatory drugs have also been reported to cause damage to the liver. Though the inhibition of members of CYP450 enzyme family has been identified as having therapeutic potential in some disease conditions, largely, their inhibition has been associated with dangerously toxic outcomes (Alaei *et al*., 2023).

A flavonoid component of the extract, 2-Hydroxychalcone, which binds strongly to members of CYP450 family, has been found to inflict damage to hepatocytes at concentrations above 20 μM through the generation of oxidative stress and down-regulation of the expression of antioxidant proteins in HepG2 cells (Qian *et al*., 2019). 2-Hydroxychalcone which also interferes with NR-PPAR-Gamma activity has been reported to reduce hyperglycemia through the NR-PPAR-Gamma pathway in diabetic rats (Eissa *et al*., 2017). The strong binding of 2-Hydroxychalcone to subunit B of Na^+/K^+-ATPase, which involves hydrophobic interactions and conventional strong hydrogen bonding, shows that the compound could be a strong inhibitor of Na^+/K^+-ATPase. Na^+/K^+-ATPase inhibitors, like cardiac glycosides (e.g., digoxin), are clinically used to enhance cardiac contractility as they regulate ion balance across the plasma membrane especially in neurons and muscle cells (Ren *et al*, 2024). 2-Hydroxychalcone could alter ion transport, which has implications for conditions such as hypertension, heart failure, or neuronal excitability, thereby offering potential as a cardioprotective agent.

The toxic effect of 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene which is shown here to bind strongly to members of CYP450 family and also to subunits in NADH dehydrogenase 1 has not been reported in the literature. The inhibition of mitochondrial NADH dehydrogenase 1 has been reported to induce significant reactive oxygen species generation which have toxic outcomes in the liver and the brain (Murali & Shivanandappa, 2022). The inhibition of NADH dehydrogenase 1 in the mitochondria by 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene may be one of the significant contributors to the cytotoxicity of *C. rostrata* epicarp extract against HeLa and MRC5-SV2 cells. This also suggests that *Cola rostrata* extract might be able to modulate energy metabolism, potentially leading to therapeutic applications in cancer cells, or side effects if inhibition of this enzyme occurs in healthy cells. The inhibition of CYP2C19 by 1-(4-methoxyphenylazo)-2-phenoxynaphthalene may invariably result in altered drug metabolism, potentially affecting the pharmacokinetics of drugs metabolized by this enzyme. The resulting modulation might be harnessed for controlling drug metabolism in individuals with CYP2C19-specific health issues.

The pharmacological activity of Anthiaergostan-5,7,9-trien-14.alpha.,15.alpha.-diol, which is one of these 10 components, in living systems has not been fully elucidated. Here, the molecular docking experiments revealed that the compound binds tightly to members of the CYP450 family and also to NADH dehydrogenase 1. The short hydrogen bond lengths (2.5–2.9 Å) indicate strong binding affinity, while hydrophobic interactions stabilize the ligands within enzyme pockets. The molecular interactions of *Cola rostrata* components with enzymes such as NADH dehydrogenase, CYP1A1, CYP2C19, and Na^+/K^+-ATPase demonstrate their potential to modulate key biological pathways which could result in toxic outcomes.

The Antioxidant Effects of Phytosterol Constituents Could Counter Toxicity

The toxic effects of the identified components of *C. rostrata* epicarp extract could be ameliorated by the rich presence of phytosterols like Stigmasterol, β-Sitosterol, γ-Sitosterol and Stigmasta-4,22-diene in the extract. These phytosterols have been shown to possess strong oxidative stress-combating activities. Koc *et al* (2021) reported that β-Sitosterol showed anti-inflammatory and anti-oxidative stress activities in rats by increasing the

activities of glutathione and the antioxidant enzyme, superoxide dismutase, while it also downregulated the levels of inflammatory proteins, tumor necrosis factor-alpha and interleukin-6. The analgesic and anti-inflammatory activity of stigmasterol was reported by Kariuki *et al* (2012), and the strong antioxidant potential of Stigmasta-4,22-dien-3-one, a closely related compound to Stigmasta-4,22-diene, was reported by Sahidin *et al* (2014). The overall toxicity of the ethanol extract of *C. rostrata* epicarp could be reduced by the presence of these steroids.

CONCLUSION

This study provides analyses of the pharmacological and toxicological profiles of components of the ethanol extract from the epicarp of *Cola rostrata* fruit. The extract contains 48 identified phytochemicals, including 1-(4-Methoxyphenylazo)-2-phenoxynaphthalene, Anthiaergostan-5,7,9-trien-14.alpha,15.alpha.-diol, and 2-Hydroxychalcone, which were evaluated for their metabolism, excretion and toxicity properties using *in-silico* techniques and molecular docking. The binding of these compounds to key metabolic proteins suggests potential effects on ion transport and liver function. However, the predicted high potential for adverse reactions, including herb-drug interactions and mitochondrial toxicity, necessitates further *in-vitro* and *in-vivo* studies to validate these findings and ensure the safe use of *Cola rostrata* in traditional medicine and drug formulations.

Authors' Contribution: **ABE**: conceptualization, data curation, formal analysis, methodology, validation, visualization, writing of original draft, review and editing of manuscript **AOS**: validation, writing of original draft, review and editing of manuscript. **OAK:** validation, review and editing of manuscript. **MBJ**: validation, review and editing of manuscript. All authors read and approved the manuscript.

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