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Pharmacological impact of secondary metabolites of *Senecio biafrae* vegetable for PPARα agonist, antioxidant, hepatoprotection, hematological, histopathological and antimicrobial potential

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Abstract

Background: This study was aimed at investigating the *in-silico* and *in-vivo* hepatoprotective, antioxidant, and antimicrobial potential of *Senecio biafrae* (Olive and Hierne) C. Jeffrey (*Asteraceae*).

Methods: The chemoinformatic assay was conducted using ligands against hepatic peroxisome proliferator-activated receptor binding protein (PPARα). Lipid examination, liver function tests, antioxidant, hematological, and histopathological examinations were studied to evaluate the hepatoprotective potential.

Results: The Total Phenolic Content (TPC) and Flavonoid Content (FC) values were 2,214 µg/mg GAE and 29.76 µg/mg QE. The hit compounds have good binding affinity and interact with the key residue of the binding site. The *in vivo* biochemical results showed a significant decrease ($p< 0.05$) in the ALT and ALP. In the lipid examination, there was a significant increase ($p< 0.05$) in serum levels of HDL (0.89±0.11), which ensures a good performance of the liver. There was significant increase ($p< 0.05$) in GSH level (3.15 ± 0.18), which shows potency in protecting the cells from toxins. The extract caused an increase in serum levels of SOD (3.15 ± 0.18) which mitigated the effect of reactive oxygen species. Increased concentration level of CAT (18.15 ±7.59) was due to the administration of the extract at 200 mg, thus regulating the cellular level of hydrogen peroxide. 400 mg of the extract led to an increase in serum levels of MDA (4.17 ± 0.71), which ensures a good performance of the liver. DPPH antioxidant IC_{50} values of the extract were 50.0 µg/mL. The highest inhibitory effect was observed against *Micrococcus varians* (17 mm).

Conclusion: This study showed that *S. biafrae* (SB) has good prospects of being a hepatoprotective drug candidate.

Keywords: *Senecio biafrae;* PPARα agonist; pharmacokinetics; antioxidant; antimicrobial.

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Background

Natural products from vegetables play a major role in primary healthcare in Africa. Secondary metabolites have an imperative pharmacological importance against some diseases and are recognized to be a major source of modern drug design and development [1-3]. Secondary metabolites provide an alternative approach to the control and treatment of diseases and health problems, due to the various side effects or resistance associated with conventional drugs [4,5]. Vegetables have been used by man from the beginning of human civilization as health remedies for human and animal diseases, because they contain moieties of therapeutic value [6,7]. Most natural products from vegetables are also used for prophylactic purposes. Recently, it has been observed on a global scale that medically, there is an increase in the use of natural products as remedies for different therapeutic purposes, and most of the secondary metabolites from vegetables have been incorporated into modern medicinal drugs [8,9]. *S. biafrae* is a naturally grown medicinal vegetable in Africa. The edible green vegetable has medicinal properties against malaria, fibromyalgia*, diabetes,* cough, arthritis, wounds, *high blood pressure*, inflammation, gastrointestinal disorders, among others [10,11]. Peroxisome proliferator-activated receptor (PPAR) α, β/δ, and γ modulate lipid homeostasis, thereby regulating lipid metabolism in the liver, the organ that largely controls whole-body nutrient/energy homeostasis, and its abnormalities may lead to hepatic steatosis, steatohepatitis, steatofibrosis, and liver cancer.[12,13] PPARα is a nuclear receptor of clinical interest as a drug target in various metabolic disorders. PPARα also exhibits marked anti-inflammatory capacities [14-16]. To the best of our knowledge, there is a paucity of information on the phytochemical and therapeutic potential of the leaf extract of *S. biafrae*. Therefore, the present study was undertaken with the aim of looking into the phytochemical analysis, PPARα agonist, antioxidant, and antimicrobial potential of the leaf extract of *S. biafrae* grown in Nigeria.

Methods

Collection and identification of the sample

The leaves of the plant were collected at Harmony Estate, Ondo, Nigeria. The plant specimen was authenticated at the University of Medical Sciences (UNIMED) herbarium as *Senecio biafrae* (*Asteraceae*) with voucher specimen number (UNIMED PBTH 051).

Preparation of leaf extract

The plant extract was prepared by the method previously used by Idowu *et al* [17]. Briefly, air-dried pulverized leaves were macerated and extracted with methanol and ethyl acetate (2:1) for at least 72 h with intermittent, shaking after which it was subjected to filtration, and the concentrated extract was kept in a refrigerator.

Evaluation of total phenolic content (TPC)

The TPC of the extract of SB was determined using Folin-Ciocalteu method. 1000 µg/mL of the extract was mixed with 1 mL of 10% Folin-Ciocalteu reagent in distilled water and then neutralized with 4 mL of 7.5% sodium carbonate solution. The sample was maintained at room temperature for 3 hrs with periodical shaking; the absorbance at 760 nm was measured using Uniscope SM 7504 UV Spectrophotometer. The index of TPC of the extract was determined as µg/mg of gallic acid equivalent (GAE) using an equation obtained from the calibration curve of gallic acid graph [18].

Evaluation of total flavonoid concentration (TFC)

The TFC of the extract of SB was determined using aluminium chloride method. Briefly, 1.0 ml of the extract, 0.10 ml of 10% aluminium chloride, 0.10 ml of sodium acetate (1 M), and 2.80 ml of distilled water. After incubation for 40 min, absorbance was measured at 415 nm using Uniscope SM 7504 UV Spectrophotometer. The index of TFC concentration is expressed as quercetin equivalents (QE) in µg per mg of extract. All assays were carried out in triplicate [19].

Evaluation of antioxidant using 2,2'-diphenyl-1-picrylhydrazil (DPPH)

The antioxidant and free radical scavenging of the extract of SB were measured by using 2,2'- diphenyl-1-picryl-hydrazyl. Briefly, the reaction mixture (3.0 ml) consists of 2.0 ml of DPPH in methanol (0.004%) and 1.0 ml of various concentrations of the extract. It was incubated for 30 min in dark, and then the absorbance was measured using Uniscope SM 7504 UV Spectrophotometer at 517 nm. The control was prepared by DPPH and methanol in place of the sample. In this assay, the positive control was ascorbic acid. The percentage of inhibition can be calculated using the formula:

$$I\% = [(A_{blank} - A_{ext})/A_{blank}] \times 100$$

Where: A_{blank} is the absorbance of the blank solution, and A_{ext} is the absorbance of the extract. The dose-response curve was plotted, and IC_{50} value for the extract and the standard was calculated [20].

Chemoinformatic pharmacological profiles

Protein retrieval and preparation

The three-dimensional (3D) crystal structure of the Peroxisome proliferator-activated receptor alpha (PPARα) was downloaded from the Protein Data Bank (http://www.rcsb.org/pdb) (PDB ID = 2ZNN)., The protein was viewed and prepared with the Protein Preparation Wizard panel of the Schrödinger Maestro suite 11.5. The preparation was carried out by filling missing loops and side chains using Prime, adding extrinsic hydrogen bonds, assigning bond orders, making disulfide bonds, producing het state at pH 7.0±2.0 using Epik, and elimination of water and other molecules used for crystallization. The protein was then optimized with PROPKA PH 7.0 and put through retrained minimization using the OPLS3 force field with hydrogen unconstrained and heavy atoms restrained to complete the protein preparation [21, 22].

Ligand preparation

The two-dimensional (2D) structures of a PPARα agonist (Fenofibrate), and 30 phytochemicals from the leaf extract of *S. biafrae* were downloaded from the NCBI PubChem database

(https://pubchem.ncbi.nlm.nih.gov) in sdf format. The structures were prepared using the LigPrep interface (Schrodinger Release 2018-1), which transforms the 2D structures into minimized 3D structures. The ligands were desalted with possible tautomers created also, low-energy states were created using OPLS3 force field, and Epik was utilized to create potential ionization states at pH 7.0±2.0 to complete the preparation process [7, 23].

Receptor grid generation

A grid box that captures the PPARα ligand binding site was generated using the Glide Grid Generation panel in Schrödinger Maestro 11.5, hence providing the site for ligand docking. The scoring grid centres the PPARα co-crystallized ligand with supplied coordinates of 10.96, 4.68, and -8.28 in the X, Y, and Z dimensions. The van der Waals (vdW) radius scaling factor of nonpolar receptor atoms and the partial atomic charge were set to 1.0 Å and 0.25, respectively [24].

Molecular docking

The prepared compounds were docked to the PPARα ligand binding site using the generated grid, through the Ligand docking panel of the Glide tool in Schrödinger Maestro 11.5. The extra precision (XP) algorithm with flexible ligand sampling was employed for the docking. The values for the partial charge cutoff (0.15) and van der Waals radii scaling factors (0.80) were at default. Similarly, the co-crystallized ligand was docked [1,25].

Molecular docking validation

The PPARα agonist co-crystallized with the protein was extracted and docked to the PPARα ligand binding site. The Root Mean Square Deviation (RMSD) of the docked poses from the native PDB pose was used to validate the docking procedure. The RMSD was calculated by making use of the "Compute RMSD to input ligand geometries" option in the output interface of Ligand docking's glide tool during docking [25].

Prime MM-GBSA

The pose file for the docked ligand- PPARα complexes was used to calculate the binding free energy (ΔG_{bind}) between the docked ligands and PPARα ligand binding site using Molecular Mechanics-Generalized Born Surface Area (MM-GBSA) panel of the Prime tool of Schrodinger 11. The OPLS3 force field, the VSGB continuum solvation model, and the minimize sampling method options were used. The Hayes and Archontis equation are a yardstick for the ΔG_{bind} calculations [26].

Pharmacokinetics

The web tools: SwissADME (http://www.swissadme.ch/) and ProTox (https://tox-new.charite.de/protoxII/) were used to investigate the pharmacokinetics, drug-likeness, and toxicity features (ADMET) of the hit compounds and the reference ligands. Each ligand's canonical SMILES was uploaded to the SwissADME and ProTox servers. The ADMET parameters were automatically generated [22,27].

Biochemical hepatoprotection, Hematological analysis, and histopathology examination

Experimental animals and treatment

Female albino rats weighing 80-120g were bought from the Anatomy Department of the University of Lagos, Lagos-State, Nigeria. The animals were kept in air-circulated cages at room temperature (28–30 °C) and received normal laboratory chow (Top Feeds, Ltd, from Lagos, Nigeria) and water ad libitum.

Induction of hepatotoxicity

A calculated dosage of diclofenac based on average body weight was injected intraperitoneally to induce hepatotoxicity in each experimental rat for two weeks. Acetaminophen was administered in groups 2-5. Group 1 (control) received no treatment, while groups, 2, 3, 4 and 5 were administered Diclofenac for seven days, after which groups 3 and 4 were treated with 200 mg and 400 mg the extract respectively, while group 5 received standard drug (Silybon 140 mg) for 14 days. The dose administration was based on previous studies [5].

Sacrifice of animals

Rats were sacrificed 24 hours after the last treatment and overnight fasting for 12 hours, before the administration of anesthesia. Blood samples for biochemical analysis were taken by puncturing the retro-orbital plexus, using Halothane and heparinized capillary tubes under ether anesthesia.

Histopathology of tissues

Liver tissues were fixed in 10% formalin, dehydrated in 95% ethanol, and then cleared in xylene before being embedded in paraffin. Sections (about 4 μm) were prepared and stained with hematoxylin and eosin (H&E) dye and examined under a light microscope by a histopathologist.

Preparation of serum

Blood drops were gently collected in plain, heparin, and ethylene diamine tetraacetic acid (EDTA). This is an anticoagulant, and this is for hematological parameters. The blood samples were put into plain and heparin plain bottles to enable clotting for about 30 minutes at room temperature, to separate the serum to be used for assay of the liver biomarkers. The serum was separated by centrifugation at 2500 rpm for 15 min and stored at -20°C until analyzed [1,5].

Biochemical assays

Lipid profile (LP), liver function tests (LFTs), antioxidant enzymes (AE), and hematological profile were studied to evaluate the hepatoprotective potential of the extract SB. The biochemical studies were carried out on the following: liver function markers; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin (TBIL). Lipid profile: high density lipoprotein (HDL), low density lipoprotein (LDL), and cholesterol (CHOL). Hematology: red blood cell count (RBC), white blood cell count (WBC), and hemoglobin (HGB).

Antioxidants: superoxide dismutase (SOD), reduced glutathione (GHS), catalase (CAT), lipid peroxidation measured through malondialdehyde (MDA) and histopathological analysis [5, 28].

Determination of in vitro antibacterial potential

The *in vitro* antibacterial potential of the leaf extract of *S. biafrae* was determined using the Agar-well diffusion assay [29]. The tested organisms were 14 clinical isolates of Gram-positive bacteria: (*Micrococcus varians* and *Staphylococcus aureus*), and Gram-negative bacteria: (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium,* and *Serratia marcescens*). Bacteria were incubated and grown overnight at 37 °C in Nutrient agar. The cultured bacteria were adjusted to 0.5 McFarland standards, 20 ml of sterilized Nutrient agar medium was homogenized and aseptically poured into sterile Petri dishes, and plates were swabbed with inocula of the test organisms and kept for 30 min. for adsorption. A sterile cork borer of 6mm in diameter was used to make uniform wells into which were added different concentrations (1000, 500, and 250 $\mu g ml^{-1}$) of the extract. The plates were allowed to stay in a refrigerator for 1 hour to allow proper diffusion of the juice solution into the medium. Synthetic antibiotic Gentamicin (10 µg per disc) was used as positive control. The plates were then incubated at 37 °C for 24 hr before visual assessment of the inhibition zones. The zone of inhibition was measured to the nearest size in millimetre (mm) using standard rule. The assay was carried out in aseptic conditions in order to achieve consistency.

Statistical analysis

All values were expressed as the mean ± S.E.M. of triplicate. Data were analyzed using one-way ANOVA, followed by the Duncan multiple range test for analysis of biochemical data using SPSS (16.0). Values were considered statistically significant at $P< 0.05$.

Results and Discussion

Polyphenol Content (PC)

The total phenolic content (TPC) of the extract was 2,214.00±0.00 µg/mg GAE. The presence of these phenolic compounds, such as *p*-vinylguaiacol, 3-pyridinecarboxylic acid, 1,6-dihydro-4-hydroxy-2-methyl-6-oxo-, ethyl ester, in the extract enhanced its pharmacological activities.

Flavonoid content (FC)

The quantitative amount of flavonoids in the leaf extract of *S. biafrae* evaluated in this study was 29.76±0.00 µg/mg QE. Eating vegetables rich in polyphenols such as flavonoids also improves memory and learning, possibly by boosting blood flow in the brain. They also protect the body's tissues against oxidative stress and associated pathologies such as cancers, coronary heart disease, and inflammation [30,31].

In vitro DPPH free radical scavenging antioxidant assay

The DPPH percentage inhibitions by the extract of SB at different concentrations (1000, 750, 500, 250 and 100 µg/mL) were 78.98, 72.28, 68.65, 62.78, and 60.33%, respectively. The extract gave an IC_{50} value of 50.0 µg/mL. This reaction occurs when the electron of nitrogen in the DPPH molecule is reduced by receiving a hydrogen atom or single electron from antioxidants [32]. The lower the IC_{50}, the higher the free radical scavenging potential [33]. Comparatively, the extract investigated in this study was observed to be a good natural radical scavenger and antioxidant agent even at very low concentrations.

Chemoinformatic pharmacological profiles results

Molecular docking

The phytochemicals from the leaf extract of SB are potential agonists of PPARα. The binding affinity, binding interaction, and binding energy were used to investigate agonistic properties of those phytochemicals by comparing them to a known agonist (Fenofibrate). The docking score from the molecular docking is equivalent to the binding affinity. The more negative the docking score, the better the binding affinity [34,35]. The docking score of our top scoring compound and the reference ligand is shown in Table 1.

The docking result of our hit compounds showed hydnocarpic acid, phytol, palmitic acid, methyl linoleate, methyl (7E)-7-hexadecenoate, methyl linolenate, and Tms score -8.752 Kcal/mol, -7.343 Kcal/mol, -7.103 Kcal/mol, -6.839 Kcal/mol, -6.692Kcal/mol, -6.519Kcal/mol, and -6.367 Kcal/mol respectively. The reference ligand (Fenofibrate) score of -8.009 Kcal/mol. Hydnocarpic acid has a higher docking score than the reference ligand, and the other top-ranking compounds have a close docking score to it (Figure 2). Thus, revealing their agonistic potential on PPARα. To validate the accuracy of the docking procedure, we computed the root mean square deviation (RMSD) calculation on the co-ligand docked pose from the native PDB pose. An RMSD value ≤ 2.0Å validates a docking procedure to be accurate [22,36]. Our analysis produces a 0.360Å RMSD (Figure 3), thus showing our docking protocol is valid and the results obtained are reliable.

We also explored the binding interaction of our top-ranking compounds and the reference ligand with the PPARα binding pocket residues (Table 1). The ligands interact with the residues through hydrogen bonds, polar, and hydrophobic interactions. The residues: Asn 219, Thr 279, Ser 280, Tyr314, Leu 331, Gly 335, His 440, Tyr 464 are the ligand binding site residues [22,37]. The hydrogen bond involving Tyr 464 is very important for maintaining the protein active conformation, and Tyr 314 is involved in PPARα selectivity [38]. Our top ligands interact with these residues. Also, the PPARα binding site was reported to be hydrophobic [39]. This can also be seen in Figure 1. Therefore, the hydrophobic properties of the ligands may contribute to their PPARα isoform selectivity and activation potential. Specifically, the reference ligand interacts with Thr 279; and hydnocarpic acid and palmitic acid interact with Ser 280, Tyr 314, His 440, and Tyr 464, which are the same residues, WY14643 (a known agonist) was reported to interact with. All together, these further support these phytochemicals as a potential agonist of PPARα. The 2D interaction diagram is shown in Figure 4.

Binding energy using MMGBSA

MM-GBSA (Molecular Mechanics-Generalized Born Surface Area) uses a more rigorous method that also takes into consideration the

solvent exposed surface of ligand to calculate the binding free energy (ΔG bind) [26]. The more negative the score, the better the ligand stability in the binding site of the protein. The result of this procedure is shown in Figure 3. Hydnocarpic acid, phytol, palmitic acid, methyl linoleate, methyl (7E)-7-hexadecenoate, methyl linolenate, and Tms score -41.69 ΔGbind, -53.44 ΔGbind, -48.49 ΔGbind, -52.96 ΔGbind, -61.56ΔGbind, -49.11ΔGbind, -15.37 ΔGbind, -52.82 ΔGbind, and -31.66 ΔGbind respectively, and the reference ligand (Fenofibrate) scores -49.63 ΔGbind. This result is shown in Figure 3. The phytol, methyl linoleate, methyl (7E)-7-hexadecenoate, and methyl dihydrohydnocarpate score higher than fenofibrate, and the others, except Tms, showed a close score. These show our compounds are stable in the PPARα ligand binding site, and as an agonist.

ADMET and the drug-likeness predictions

The absorption and distribution properties of the compounds reveal (Table 2) that all the compounds except Tms and phytol possess high gastrointestinal absorption properties, that is, all but these two can be absorbed in high amounts into the blood to circulate to target organs, through the intestine when taken through the oral means. It also reveals that, except phytol, all compounds are not substrates for P-Glycoprotein, a multidrug resistant protein 1 (MDR1) that extrudes a substrate-drug out of the cell, thus preventing them from reaching their target. The BBB Permeant predicts if a compound can cross the blood-brain barrier to have a neuro-effect, such as a neuroprotective or neurotoxic effect on the brain. The result showed that the phytol, methyl linoleate, and Tms are not permeant of the BBB, while the rest of our top-ranking compounds are permeants. Further research can be considered to understand the specific roles of these compounds in the brain.

Drug metabolism properties of our compounds reveal only Tms to show no inhibition of any of the CYP450 enzymes, while the others show few inhibitions, and the reference ligands inhibit four of the five CYP450 enzymes (Table 3). Drugs that inhibit CYP450 enzyme such as CYP3A4, bioaccumulates, are likely to cause Drug-Drug interactions-One drug administered significantly alters the plasma concentration, half-life, or toxicity of another drug. It also leads to slow down in the metabolism of substrates, with typical increased drug effects. The no-inhibition of Tms shows it can be easily metabolized by the body, and the inhibition showed by the other compounds reveals potential hindrances in their metabolism, although with a beneficial increased half-life, but has potential for toxicity.

All investigated compounds are drug-like since they obey the Lipinski rules, which state that drug-like compounds should not violate more than one of the rules (drug-like compounds should have MW < 500, HBD < 5, HBA < 10, Logp ≤ 5) (Table 4). Polar Surface Area (PSA) greater than 140 Å2 reduces oral bioavailability and cell membrane permeability. The TPSA (≤140 $Å^2$) value of the studied compounds ranges from 20.23 Å2 – 52.6 Å2. The lower than 140 Å2 TPSA of the compounds, therefore, means they will be available in the system. Similarly, a bioavailability score of 0.55 or higher for the compounds reveals their availability in the system. The toxicity prediction result is in Table 4. Tms from our hit compound is strongly toxic and can be immunotoxic and mutagenic. Hydnocarpic acid is slightly toxic while the other ligands are non-toxic. The reference ligands show properties for carcinogenicity. The results were predicted with an accuracy of at least 69%, revealing that our results are reliable.

The "toxicity class" predicts acute toxicity. The LD_{50} is the dose predicted to kill 50% of a population. Acute toxicity increases with decreasing LD_{50}. Methyl linolenate and methyl linoleate, with 20000 mg/kg LD_{50}, show no toxicity at all, as revealed with a toxicity class of 6. Hydnocarpic acid shows slight toxicity with a toxicity class of 2 and 48 LD_{50}; while phytol, palmitic acid, methyl (7*E*)-7-hexadecenoate, and the reference ligands show non-toxic properties with the toxicity class ranging from 4-5 and LD_{50} 900-3450 mg/kg. The Log *k*p (Skin permeation coefficient) in Table 2. Predicts skin toxicity potential by indicating. The compound's ability to penetrate the skin. Negative value shows that it does not readily penetrate the skin, and permeation potential reduces with increasing negative value. The compounds' negative Log *k*p values that range from -4.83 cm/s to -2.29 cm/s, therefore show that the compounds cannot permeate the skin to cause toxicity.

Biochemical parameters

The evaluation of high-density lipoprotein (HDL) results (Table 5) showed that there was significant decrease ($p<0.05$) in serum level of HDL for diclofenac only induced group (1.02±0.36) when compared to control group (1.27±0.26), indicating a liver disease. Meanwhile, there was a slight decrease ($p<0.05$) in the HDL level for the SB 200 mg (0.89±0.10) when compared to the control group. In SB 400 mg (1.21±0.37), there was an increase ($p<0.05$) in the serum level of HDL when compared to Diclofenac only induced group. Also, there was a decrease ($p<0.05$) in HDL level in the Silbon (1.05±0.20) treated group in comparison with the control group. The discovery of low levels of HDL enzymes in Diclofenac only- induced rats serum indicated that the liver was damaged to an extent and also that skeletal muscle was also damaged. Significant increase shown in the treated group with the extract at 400 mg proves the potency of SB has hepato-protective potential.

It was also observed in Table 5 that there was significant ($p<0.05$) increase in serum cholesterol (CHOL) level in Diclofenac only induced rats when compared to control group. For the sample investigated, there was slight ($P<0.05>$) reduction in CHOL in the extract treated group when compared to Diclofenac only induced rats. Likewise, there was slight ($P<0.05$) increase in serum CHOL in extract treated group at a dose of 200 mg/kg (2.04±0.30) when compared to control group (1.89±0.30). At a dose of 400 mg/kg, there was significant ($P<0.05$) reduction in serum CHOL in the extract treated group (1.67±0.15) when compared to control group. There was a significant ($P<0.05$) decrease in serum CHOL level in Silbon 140 mg (1.95±0.11) treated rats compared to Diclofenac only (2.28±0.08) induced rats. The SB extract at a dose of 200 mg/kg showed a significant ($P<0.05$) decrease in serum CHOL level compared to Diclofenac only induced rats. The SB extract at a dose of 400 mg/kg showed a significant ($P<0.05$) decrease in serum CHOL level compared to Diclofenac only induced group.

For the evaluation of low-density lipoprotein (LDL), according to Table 5, there was an increase ($p<0.05$) in the LDL cholesterol level for the Diclofenac only (0.95±0.151) induced group when compared to the control group (0.70±0.15), which indicates a disease. The LDL cholesterol level for the extract treated group at 200 mg is 0.79 mg/dl, and this shows a decrease ($p<0.05$) when compared to Diclofenac only induced group. Moreover, there was also a marked ($p<0.05$) reduction in the serum levels of LDL in the 400 mg (0.65±0.08) extract group when compared to the Diclofenac only induced group. The LDL cholesterol level for the Silbon 140 mg treated group is 0.56 mg/dl. This shows a ($p<0.05$)

reduction when compared to the control group's LDL cholesterol level, indicating a decrease.

Table 6 showed that the SGOT (AST) level for the Diclofenac induced group is 152.63 mg/kg. This is higher than the control group's level (134.43 mg/kg), indicating an increase. The serum level of SGOT (AST) in the extract treated group (SB 200 mg/kg) is 122.70 mg/kg; this shows a decrease ($p < 0.05$) when compared to the Diclofenac group. There was a slight decrease ($p < 0.05$) in the SGOT (AST) level for the extract group treated at 400 mg (144.37 mg/kg) in comparison to the Diclofenac group. The SGOT (AST) level for the Silbon 140 mg group is 131.33 mg/kg. This is slightly higher ($p<0.05$) than the control group's level, indicating a slight increase. It was clearly observed in Table 6 that there is an increase ($p<0.05$) in SGPT (ALT) levels for the Diclofenac induced 76.50 and Silbon 140 mg (64.47) treated groups compared to the control group (51.87). There was an increase ($p<0.05$) in the serum SGPT (ALT) level in the Diclofenac treated group when compared to the control group. There was a significant decrease ($p<0.05$) in SGPT (ALT) level of the extract treated group at 200 mg (51.70) and 400 mg (56.20), when compared to Diclofenac induced group. Treatment with SB extracts at 200 mg and 400 mg attenuated ALT levels.

According to results in Table 6, there is a substantial increase ($p<0.05$) in ALP levels for the Diclofenac only (735.77) induced and Silbon 140 mg (514.43) groups, compared to the control group (362.90). There was a significant increase ($p<0.05$) in ALP level for the Diclofenac only induced group than the control group's level, indicating a substantial increase. The SB 200 mg (384.83) group shows a significant decrease ($p<0.05$) in ALP level than the Diclofenac only induced rats. There was also a slight reduction ($p<0.05$) in the ALP levels in the extract group SB at 400 mg (409.73), in comparison to the Diclofenac only group. Elevated ALP levels may suggest liver or bone conditions, among other potential causes, while the decrease in the ALP levels of the extract treated rats was as a result of the amelioration property of the extract. There was an increase ($P<0.05$) in TBIL levels for the Diclofenac only induced group (3.13), compared to the control group (2.33). There was significant increase ($p<0.05$) of SB 200 mg (1.87) extract, when compared with other experimental groups.

The extract at 400 mg (2.43) gave a rise ($P<0.05$) in the TBIL levels than the control, also in the Silbon 140 mg group (2.80), there was a significant increase ($P<0.05$) serum level of TBIL. The SB extract at 200 and 400 mg gave remarkable decrease ($P<0.05$) in TBIL levels when compared to Diclofenac only induced group (Table 6). Elevated TBIL levels may suggest liver or gallbladder issues.

In the antioxidant enzymes assay, there was significant increase ($p< 0.05$) in group 4 of GSH level (3.15±0.18), which shows potency in protecting the cells from toxins. Antioxidant markers are presented in Table 7. The GSH results revealed that, Diclofenac only group (7.57±1.14) does not decrease GSH levels compared to the control group (7.57±0.00), which implies that it does not suppress glutathione. Both the varying doses of the extracts at 200 mg/kg (8.78±1.26) and 400 mg (8.78±0.78) increased the level of GSH, revealing protective effects and antioxidant potential, this is however comparable to the standard treatment 140 mg Silbon + diclofenac (9.15± 1.89). The absence of dose-dependent response shows that 200 mg is just as effective as 400 mg in regulating the level of GSH, mitigating oxidative stress and toxicity. The GSH results revealed non-significant ($p > 0.05$) increase in the induced rat's serum with diclofenac only (7.57±1.14), compared with the control (7.57±0.00). Significant increase ($p < 0.05$) was observed in the varying doses of extract at 200 mg (8.78±1.26), 400 mg (8.78±0.78) and 140 mg Silbon + diclofenac group, which is the standard drug treated group (9.15± 1.89) when compared with the diclofenac only group. There was non-significant ($p > 0.05$) increment between extract varying doses at 200 mg and 400 mg/kg SB groups.

According to Table 7, the 200 mg of the extract caused an increase in serum levels of SOD (3.15±0.18), which mitigated the effect of reactive oxygen species. The SOD results revealed that, Diclofenac only (4.13±1.03) shows non-significant ($p > 0.05$) decrease in SOD level, indicating decline in antioxidant, when compared to the control group (4.56±0.00). There was significant ($p < 0.05$) decrease in the SOD level at the administration of the varying dose of the extracts at 200 mg (3.96±1.15), 400 mg (3.15±0.18) and 140 mg Silbon + diclofenac group (3.86±0.37), which is the standard treatment. Notably, the lower dose of the extract (200 mg) result shows higher SOD level, implying that the SB extract does not enhance the SOD activity, in fact more suppressive effects is observed at higher dose (400 mg) of the extract. This however has inhibitory effects on SOD, resulting to reduction in antioxidant enzyme activities, consequently increasing oxidative stress and cell damage.

Increased concentration level of CAT (18.15±7.59) as shown in Table 7 was due to the administration of the extract at 200 mg, thus regulating the cellular level of hydrogen peroxide. 400 mg of the extract led to an increase in serum levels of MDA (4.17±0.71), which ensures a good performance of the liver. The CAT result shows that, Diclofenac only group (21.18±5.69) slightly decreased in CAT level, which shows minimal impact of the diclofenac compared to the control group (21.94±0.00). There is significant ($p < 0.05$) decrease in CAT level in both varying doses of the extract at 200 mg (18.15±7.59), 400 mg (18.15±7.59) and 140 mg Silbon + diclofenac group (19.58±0.65), which is the standard treated group. The absence of difference between SB 200 mg and SB 400 mg implies no dose-dependent effects. The optimal dose should be 200 mg of SB, because there was no additional decrease in catalase activity at 400 mg, due to the enzyme inhibition reaching a particular limit, 200 mg precisely. Enzyme inhibition reaching its limit indicates that CAT could no longer break down hydrogen peroxide effectively, which can lead to the accumulation of H_2O_2 eventually leading to oxidative stress and DNA damage. The MDA result (Table 7) shows that, both Diclofenac only (3.44±0.47) and control group (3.44±0.00) gave the same result, there was no change from the control, and this implies that diclofenac did not increase lipid peroxidation. There was significant increase in MDA at 200 mg (4.25±0.40), 400 mg (4.17±0.71) and 140 mg Silbon + diclofenac group (3.74±0.02), in comparison to Diclofenac only group, indicating that SB extracts at both doses increase lipid peroxidation and oxidative stress. However, 400 mg proved to be more potent by reducing the MDA level compared to 200 mg, which implies that the lipid peroxidation and oxidative stress had been reduced. Lipid peroxidation leads to inflammation, cell membrane damage and disease progression.

Hematology

From Table 8, the WBC count for the Diclofenac only induced group is 7.67 cells/µL, which is slightly higher than the control group's count (7.47), indicating a small increase; the WBC count for the 200 mg extract in rats' serum is 8.30 cells/µL. This is higher than both the control group and the Diclofenac only group, indicating an increase. The WBC count for the extract treated group at 400 mg is 8.50 cells/µL, which is higher than both the control group and the Diclofenac only group, indicating an increase. The WBC count for the Silbon 140 mg group is 6.33 cells/µL. This is lower than the control group's count, indicating a decrease. White blood cell counts are indicators of the body's immune response and overall health. Induction of Diclofenac only gave reduction in WBC concentration which is a strong indication of inflammation in the body.

The HGB level for the Diclofenac only group is 13.37 mg/dl, which shows a significant increase ($p<0.05$) than the control group's level (11.17), indicating an increase (Table 8). The HGB level for the extract treated (200 mg) group is 10.93 mg/dl, which has significant decrease ($p<0.05$), when compared to the Diclofenac only induced and the Control group's serum level, indicating a decrease. In the SB 400 mg (10.03 mg/dl) extract treated group, the HGB level was significantly decreased ($p<0.05$), when compared to both the control and Diclofenac only group. The Hb level for the Silbon 140 mg group is 12.57 mg/dl. This is higher than the control group's level, indicating an increase. Hemoglobin levels are important indicators of the oxygen-carrying capacity of the blood.

As reported in Table 8, the RBC count was 7.85 cells/µL in the Diclofenac only induced group. This indicates an increase in comparison to the control group. There was a significant decrease in the RBC level in the 200 mg extract treated group, compared to Diclofenac only group. There was also a significant decrease in 400 mg extract group, compared with Diclofenac only induced group. The extract groups and Silbon 140 mg groups show no significant change in RBC counts compared to the control group. Red blood cell counts, are important indicators of the blood's oxygen-carrying capacity.

Histopathological Result

The result of histopathology indicates a degeneration of normal configuration of liver cells and infiltration of lymphocytes in the diclofenac induced group. The extract at dose of 400mg/kg reveals a cellular protection with clear veins and organized sinusoidal spaces, showing that *S. biafrae* has more recovery effects on damaged liver cells (Plates 1-5).

Histological illustration of rat liver sections stained with H and E, ×400.

Plate 1: Control showing no lesions or abnormalities.

Plate 2: Diclofenac only: Illustration of marked and severe vacuolar degeneration (fatty degeneration) of hepatocytes (arrow) (x400; H & E).

Plate 3: Diclofenac and SB extract (200 mg/kg body weight): Illustration of moderate vacuolar degeneration of hepatocytes.

Plate 4: Diclofenac and SB extract (400 mg/kg body weight): Illustration of mild vacuolar degeneration of hepatocytes.

Plate 5: Diclofenac and Silbon (140 mg/kg): Illustration of mild vacuolar degeneration of hepatocytes.

Antibacterial activity

The leaf extract exhibited wide range of inhibitory effects against the isolates, where *M. varians* showed highest zone of inhibition (Table 9). This is followed by Gram-negative: *E. coli* (13 mm), *K. pneumonia* (14 mm), *P. aeruginosa* (12 mm), *P. mirabilis* (16 mm), *S. marcescens* (14 mm), *S. typhimurium* (15 mm) and then Gram-positive: *M. varians* (17 mm) and *S. aureus* (12 mm). The extract had a good potential for therapeutic use against the bacterial pathogens and therefore can be applied in treating bacteria disorders. The bioactivity of the sample is due to the synergetic action of the phytochemicals in it, most especially polyphenols, terpenoids etc. Natural antibiotics are good alternative therapy for common diseases in place of more invasive synthetic antibiotics that have residual side effects after use. Most vegetables have natural antibiotic properties. They help support a healthy immune system and make a healthy addition to a balanced diet [40].

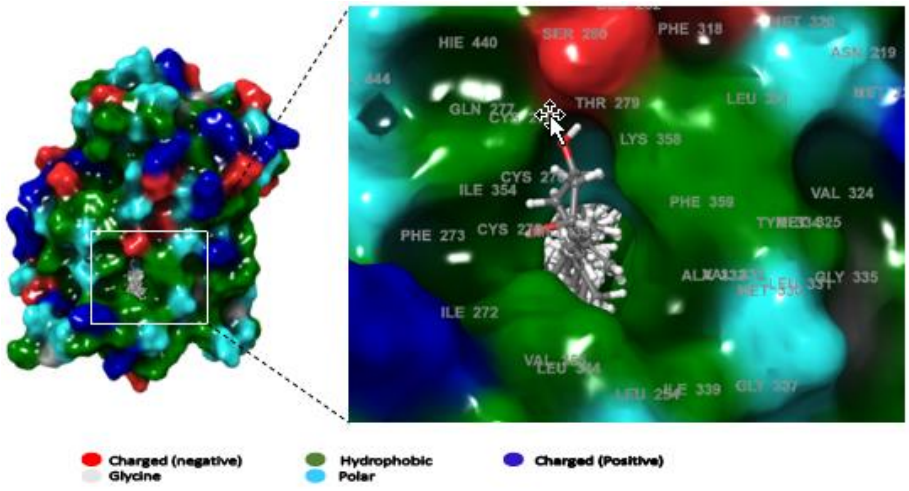


Figure 1. The 3D view of the Peroxisome proliferator activated receptor alpha (PPARα) showing the bound ligands, and the site residues. The legend shows the Residue property.

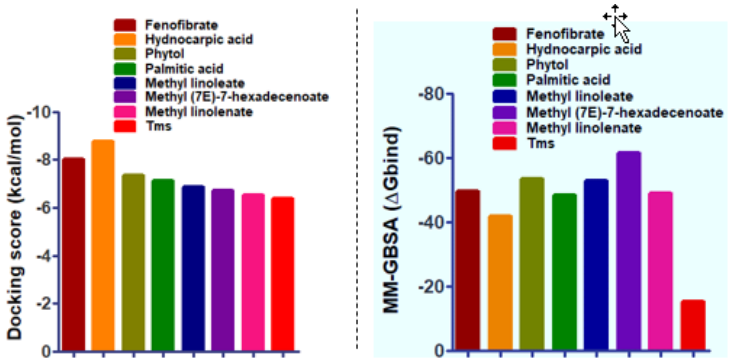


Figure 2. Graphical representations of the docking scores (left), and binding free energy change (Right-blue) of the reference ligand (Fenofibrate) and top 7 hit compounds of Senecio biafrae on PPARα binding site.
Tms is (5E,7E)-25-[(trimethylsilyl)oxy]-9,10-secocholesta-5,7,10-triene-1,3-diol

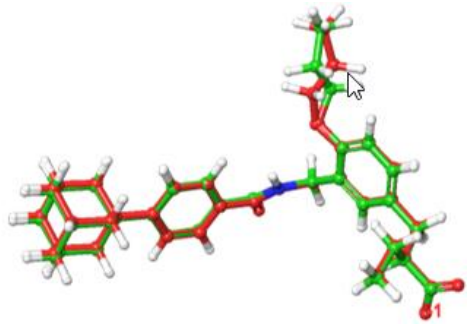


Figure 3. The superimposition of the native co-ligand poses, and the docked co-ligand pose on PPARα (RMSD is 0.360Å).
Red is docked pose, Green is native PDB pose.

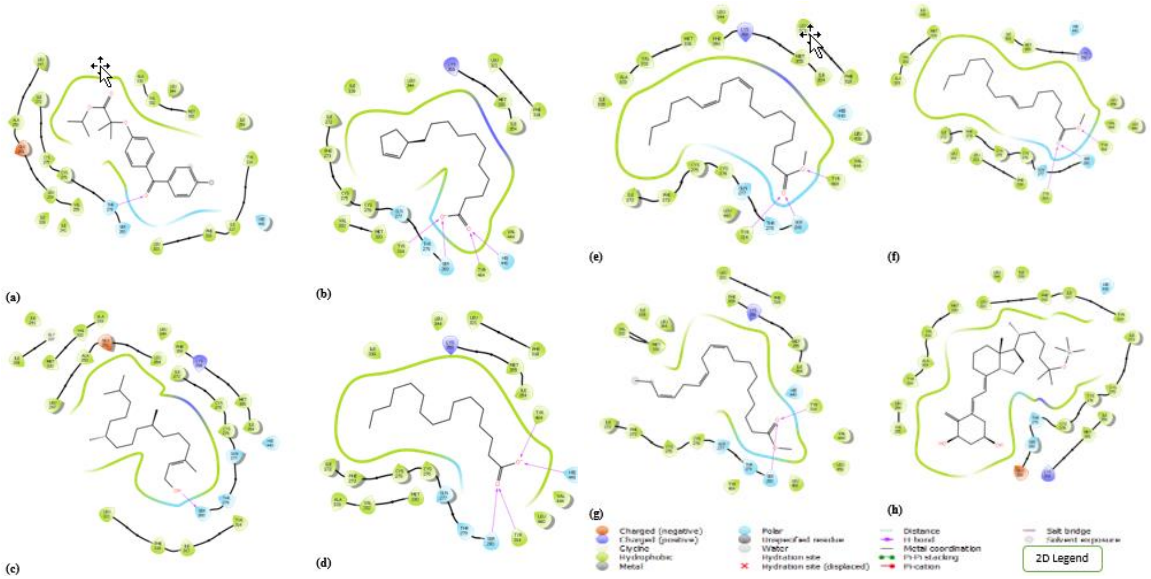


Figure 4. The 2D interaction diagram of the top hit ligands and reference ligands in the active site of PPARα with their interaction legend.
(a) Fenofibrate, (b) Hydnocarpic acid, (c) Phytol, (d) Palmitic acid, (e) Methyl linoleate, (f) Methyl (7E)-7-hexadecenoate, (g) Methyl linolenate (h) Tms. Fenofibrate is the reference ligand

Photomicrographs of female albino rat liver

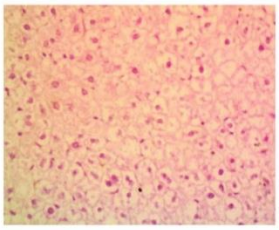


Plate1: Stained cross section of the liver of (Control group) female albino rats without treatment. No pathological changes were observed. (H and E Stain (×400).

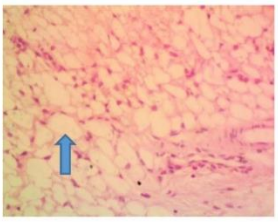


Plate 2: Photomicrograph of a section of liver from female albino rats in Diclofenac only group. (H and E stain and magnification of x400).

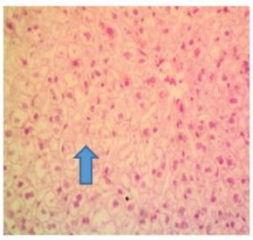


Plate 3: Photomicrograph of a section of liver from female albino rats that were administered with Diclofenac and SB extract (200 mg/kg) using H and E stain and magnification of x400.

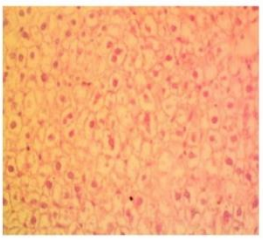


Plate 4: Photomicrograph of a section of liver from female albino rats that were administered with Diclofenac and SB extract (400 mg/kg) using H and E stain and magnification of x400.

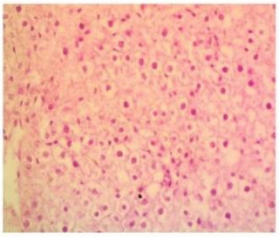


Plate 5: Photomicrograph of a section of liver from female albino rats that were administered with Diclofenac and Silbon 140 mg/kg using H and E stain and magnification of x400.

Plate 1-5. Histopathology of the liver

Table 1. 2D Structure, docking score, and PPARα residues-ligand interaction of the top ten (10) ranking compounds from *S. Biafrae* and reference ligands

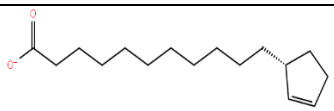
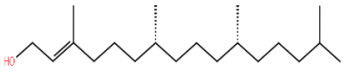
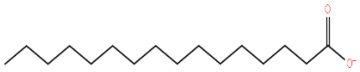
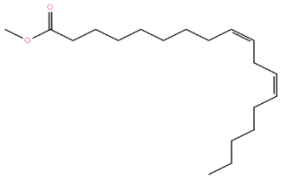
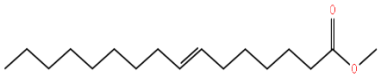
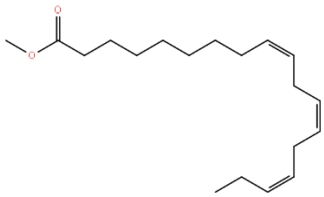
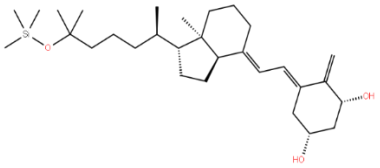
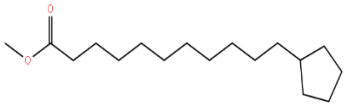
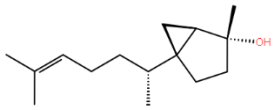
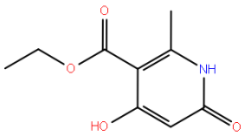
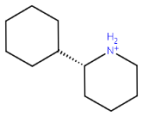
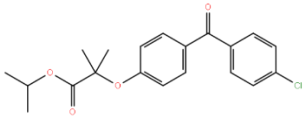
Active compounds	2D Structures	Docking scores (kcal/mol)	MMGBSA dG Bind	H-bond with PPARα
Hydnocarpic acid		-8.752	-41.69	Ser 280 Tyr 314 His 440 Tyr 464
Phytol		-7.343	-53.44	Ser 280
Palmitic acid		-7.103	-48.49	Ser 280 Tyr 314 His 440 Tyr 464
Methyl linoleate		-6.839	-52.96	Ser 280 Tyr 314
Methyl (7E)-7-hexadecenoate		-6.692	-61.56	Ser 280 Tyr 314 Tyr 464
Methyl linolenate		-6.519	-49.11	Ser 280 Tyr 314
(5E,7E)-25-[(trimethylsilyl)oxy]-9,10-secocholesta-5,7,10-triene-1,3-diol		-6.367	-15.37	-
Methyl dihydrohydnocarpate		-6.316	-52.82	Tyr 314 His 440 Tyr 464
Cis-sesquisabinene hydrate		-6.113	-31.66	Tyr 464 His 440
Ethyl 4-hydroxy-2-methyl-6-oxo-1,6-dihydro-3-pyridinecarboxylate		-5.979	-38.28	Asn 219 Leu 331 Gly 335
2-cyclohexylpiperidine		-5.97	-30.28	-
Fenofibrate*		-8.009	-49.63	Thr 279

Table 2. Prediction of the pharmacokinetic properties of the hit compounds and the Reference Ligand by Swiss ADME

Compounds	GI abs	BBB Perm	P-gp sub	CYP1A2 inhibitor*	CYP2C19 inhibitor*	CYP2C9 inhibitor*	CYP2D6 inhibitor*	CYP3A4 inhibitor*	Log *kp* (cm/s)
Fenofibrate	High	+	-	+	+	+	+	-	-4.83
Hydnocarpic acid	High	+	-	+	+	+	-	-	-3.65
Phytol	Low	-	+	-	-	+	-	-	-2.29
Palmitic acid	High	+	-	+	-	+	-	-	-2.77
Methyl linoleate	High	-	-	+	-	+	-	-	-3.25
Methyl (7E)-7-hexadecenoate	High	+	-	+	-	-	-	-	-3.41
Methyl linolenate	High	+	-	+	-	+	-	-	-3.62
Tms	Low	-	-	-	-	-	-	-	-4.29
Methyl dihydrohydnocarpate	High	+	-	+	-	-	-	-	-2.92
Cis-sesquisabinene hydrate	High	+	-	-	+	+	-	-	-4.76

Tms is (5E,7E)-25-[(trimethylsilyl)oxy]-9,10-secocholesta-5,7,10-triene-1,3-diol. + denotes Yes, - denotes No. GI abs- gastrointestinal absorption. BBB perm- Blood-brain barrier permeant. P-gp sub- P-Glycoprotein substrate. *Cytochrome p450 enzyme isoforms inhibition. Log *k*p -Skin permeation.

Table 3. Prediction of the drug likeness properties of the hit compounds and the Reference Ligand by Swiss ADME

Compounds	MW (g/mol)	HBD	HBA	TPSA (Å²)	C. Logp	Bio. Sco.	Lipinski violation*
Fenofibrate	360.83	0	4	52.6	4.4	0.55	0
Hydnocarpic acid	252.39	1	2	37.3	4.49	0.85	0
Phytol	296.53	1	1	20.23	6.22	0.55	1
Palmitic acid	256.42	1	2	37.3	5.2	0.85	1
Methyl linoleate	294.47	0	2	26.3	5.69	0.55	1
Methyl (7E)-7-hexadecenoate	268.43	0	2	26.3	5.22	0.55	1
Methyl linolenate	292.46	0	2	26.3	5.55	0.55	1
Tms	488.82	2	3	49.69	6.18	0.55	1
Methyl dihydrohydnocarpate	268.43	0	2	26.3	5.22	0.55	0
cis-sesquisabinene hydrate	222.37	1	1	20.23	3.81	0.55	0

*is the number of Lipinski rules violated. The rule states that druglike compounds should have Molecular weight (MW) <500, Hydrogen Bond Donor (HBD) < 5, Hydrogen Bond Acceptor (HBA) < 10, and Octanol/Water partition coefficient (logP) ≤ 5. Topological polar surface area (TPSA). Bioavailability Score (Bio. Sco.) Consensors Logp (C. logp)

Table 4. ProTox toxicity prediction of the hit compounds and the reference ligand

Compounds	HT	CG	IT	MG	CT	Toxicity class*	PA (%)	LD_{50} (mg/kg)
Fenofibrate	-	+	-	-	-	4	100	1600
Hydnocarpic acid	-	-	-	-	-	2	72.9	48
Phytol	-	-	-	-	-	5	100	5000
Palmitic acid	-	-	-	-	-	4	100	900
Methyl linoleate	-	-	-	-	-	6	72.9	20000
Methyl(7E)-7-hexadecenoate	-	-	-	-	-	5	72.97	3000
Methyl linolenate	-	-	-	-	-	6	72.9	20000
Tms	-	-	+	+	-	1	69.26	1
methyl dihydrohydnocarpate	-	-	-	-	-	5	72.9	5000
cis-sesquisabinene hydrate	-	-	-	-	-	5	70.97	3450

Tms is (5E,7E)-25-[(trimethylsilyl)oxy]-9,10-secocholesta-5,7,10-triene-1,3-diol. + denotes active, - denotes inactive. HT-Hepatotoxicity, CG-Cytogenicity, IT-Immunotoxicity, MG-Mutagenicity, CT-Cytogenicity, PA-Prediction Accuracy. *value 1 is toxic, 3 is moderately toxic, and value 6 is strongly non-toxic.

Table 5. The effect of diclofenac, SB extract and silbon140 mg (standard drug) on Lipid

GROUP	HDL (mg/dl)	CHOL (mg/dl)	LDL (mg/dl)
1	1.27± 0.26	1.89±0.30	0.70±0.15
2	1.02± 0.36	2.28±0.08	0.95±0.15
3	0.89±0.10	2.04±0.30	0.79±0.11
4	1.21± 0.37	1.67±0.15	0.65± 0.08
5	1.05± 0.20	1.95±0.11	0.56±0.25

Table 6. The effect of Dichlofenac, SB extract and Silbon 140 mg (standard drug) on liver function markers

Group	SGOT (AST) (IU/L)	SGPT (ALT) (IU/L)	ALP (IU/L)	TBILI (IU/L)
1	134.43±7.97	51.87±5.76	362.90±92.51	2.33±0.26
2	152.63±3.19	76.50±3.19	735.77±76.87	3.13±0.55
3	122.70±5.20	51.70±6.33	384.83±22.87	1.87±0.08
4	144.37±17.40	56.20±5.60	409.73±11.75	2.43±0.35
5	131.33±7.04	64.47±6.08	514.43±49.72	2.80±0.50

Table 7. The effect of Dichlofenac, SB extract and Silbon 140 mg (standard drug) on antioxidant parameters

Group	GSH (mg/dl)	SOD (mg/dl)	CAT (mg/dl)	MDA (mg/dl)
1	7.57±0.00	4.56±0.00	21.94±0.00	3.44±0.00
2	7.57±1.14	4.13±1.03	21.18±5.69	3.44±0.47
3	8.78±1.26	3.96±1.15	18.15±7.59	4.25±0.40
4	8.78±0.78	3.15±0.18	18.15±7.59	4.17±0.71
5	9.15± 1.89	3.86±0.37	19.58±0.65	3.74±0.02

Table 8. The effect of Dichlofenac, Extract and Silbon 140 mg (standard drug) on Hematology

Group	WBC (mg/dl)	HGB (mg/dl)	RBC (mg/dl)
1	7.47±2.11	11.17±0.97	6.33±0.58
2	7.67±1.03	13.37±0.32	7.85±0.13
3	8.30±1.82	10.93±0.64	6.78±0.62
4	8.50±1.50	10.03±1.82	7.38±0.53
5	6.33±2.74	12.57±1.13	7.38±0.53

Table 9. Zones of inhibition (mm) showing the bactericidal properties of the leaf extract of *S. biafrae*

Conc. (µg/ml)	Leaf Extract			Synthetic Antibiotic
Organisms	17-12 mm			GEN
	1000	500	250	10µg
***E. coli* (-)**	13	13	13	15
***K. pneumonia* (-)**	14	14	12	20
***M. varians* (+)**	17	15	15	20
***P. aeruginosa* (-)**	12	12	12	11
***P. mirabilis* (-)**	16	16	12	18
***S. agalactiae* (+)**	-	-	-	20
***S. aureus* (+)**	12	12	12	20
***S. marcescens* (-)**	14	14	14	17
***S. typhimurium* (-)**	15	12	10	20

Resistant (–), not sensitive (<8 mm), sensitive (9–14 mm), very sensitive (15–19 mm) and ultrasensitive (>20 mm)

Conclusion

This study showed that the phytochemicals in *S. biafrae* are pharmacologically active. The chemoinformatic results indicate that hit compounds investigated in this study have better and close binding affinity to PPAR-α compared to Fenofibrate. The safety profile characterized our hit compounds as less toxic in addition to their drug-likeness properties. The secondary metabolites in the sample exhibited combinatorial potential for drug development. The hit compounds have good binding affinity and interact with the key residue of the binding site. Moreover, our results suggest that phytochemicals in the sample are potential PPARα agonists. From the chemoinformatic study, the inhibitory potential of phytochemicals using molecular docking and Molecular Mechanics-Generalized Born Surface Area (Prime MM-GBSA), hydrocarpic acid, phytol, palmitic acid, and methyl linoleate has a docking score of -8.752 Kcal/mol, -7.393 Kcal/mol, -7.103 Kcal/mol, and -6.839 Kcal/mol respectively; while the known inhibitor, Ischemin (positive control) has a docking score of -5.444 Kcal/mol. The *in vivo* biochemical results showed significant decrease ($p< 0.05$) in the ALT and ALP of group 3 showing the protective effect of the extract on hepatocytes. The result of histopathology indicates a degeneration of normal configuration of liver cells and infiltration of lymphocytes in the diclofenac induced group. The extract at dose of 400mg/kg reveals a cellular protection with clear vein and organized sinusoidal spaces showing that *S. biafrae* has more recovery effects on damaged liver cells. Enzymes in the extract at 200 mg and 400 mg are an indication of protective potency and production of red blood cells. The extract caused an increase in serum levels which mitigated the effect of reactive oxygen species. Enzymes in the sample is an indication of protective potency and production of red blood cells. The result of histopathology indicates a degeneration of normal configuration of liver cells and infiltration of lymphocytes in the diclofenac induced group. The extract at dose of 400mg/kg reveals a cellular

protection with clear veins and organized sinusoidal spaces showing that *S. biafrae* has more recovery effects on damaged liver cells. These results showed that the sample investigated is a potential source of dietary antioxidant, antimicrobial agents and demonstrate the importance of this plant in medicine and in assisting primary health care in this part of the world. The study showed that *S. biafrae* has good prospects of being hepatoprotective drug candidate. The hepatoprotective potential of extract has been encouraging. The extract had a good potential for therapeutic use against liver disorders, reactive oxidative stress (ROS) problems, bacterial pathogens. This study showed that *S. biafrae* has good prospects of being natural drug candidate.

Abbreviations

GAE: Gallic acid equivalent
QE: Quercetin equivalent
LP: Lipid profile
LFTs: liver function tests
AE: antioxidant enzymes
ALT: alanine amino transferase
AST: aspartate amino transferase
ALP: alkaline phosphate
TBIL: total bilirubin
HDL: Lipid profile; high density lipoprotein
LDL: low density lipoprotein
CHOL: cholesterol
RBC: red blood cell count
WBC: white blood cell count
HGB: hemoglobin
SOD: superoxide dismutase
GHS: reduced gluthathione
CAT: catalase
MDA: malondialdehyde
LD_{50}: Lethal dose 50
CYP450: Cytochrome P450

Authors' Contribution

ZSO: Contributed to conceptualization, design, provided recourses, investigation, data collection, analyses, writing, editing and approved the final manuscript. MTAO, CAA, JRA, AIA, OFO, OTO and ACO: Contributed equally as the members of the research team in investigation, data collection, analyses, writing, edited and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest

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