**Original Research Article**

**Bioavailability, pharmacokinetics, drug-likeness and Toxicity Predictions of Hexane Fraction of *Catunaregam nilotica root-bark extract* using Swiss ADME and PRO TOX II**

**ABSTRACT**

*Plant-based products are recognized as sources of therapeutic agents. Catunaregam nilotica, traditionally used in medicine, contains bioactive compounds that warrant further investigation. This study predicted the physicochemical, pharmacokinetics and toxicological properties of compounds identified from the n-Hexane fraction of Catunaregam nilotica root-bark extract using in-silico tools. Components of the n-Hexane fraction were identified using GS-MS analysis. Online server SwissADME was employed for physicochemical and pharmacokinetics predictions of the identified compounds, while ProTox-3.0 evaluated the possible toxicity of the compounds. The identified compounds' drug-likeness was determined using the Lipinski rule of five (Ro5). The results of GC–MS analysis indicated a total of seven compounds in the fraction*. *The major bioactive constituent was found to be 9-Octadecenoic acid (66.50%)*. *The result of the physicochemical properties showed that all the compounds, except 1-(hydroxymethyl)-1,2-ethanediyl ester adhered to Ro5. None of the identified compounds inhibited CYP2D19 or CYP3A4 upon assessment of their inhibitory effects profile in several cytochromes P450 isoforms. In contrast, all the compounds inhibited CYP1A2, while some inhibited CYP2C9. All compounds satisfied the drug-likeness evaluation, except 1-(hydroxymethyl)-1,2-ethanediyl ester and 9-Tetradecenal. Approximately 60% of the compounds were non-mutagenic and non-carcinogenic which establishes their safety. Overall, the in-silico data suggest that these compounds exhibit favorable physicochemical and pharmacokinetic profiles, supporting their potential as safe candidates for drug development. Future work should focus on experimental validation of the in-silico predictions and exploring the pharmacological efficacy and safety of the identified compounds through in-vitro and in-vivo studies.*

**Keywords: Bioavailability, Pharmacokinetic, Drug-likeness, Toxicity.**

**1.0 Introduction**

Natural products (NPs) are a valuable source of therapeutic options due to their ability to generate a wide range of bioactive metabolites with complex synthesis scaffolds (Duran-Iturbedi et al., 2020). While they exhibit a diverse array of biological activities, some may have toxicity concerns. Interestingly, nearly 40% of all approved drugs have roots in NPs or have been influenced by them, a percentage that has risen to 50% as of 2014. This includes drugs derived from synthetic or semisynthetic derivatives and drugs that have taken inspiration from natural sources (Duran-Iturbedi, Barbara, & Medina-Franco, 2020).

Introducing a new drug to the pharmaceutical market often faces challenges related to its efficacy and safety. These challenges can be due to the drug's absorption, distribution, metabolism, excretion (ADME) properties, and toxic effects (Patrick & Vladimir, 2019). However, assessing these properties using modern methods can be expensive, time-consuming, and labour-intensive. Additionally, these studies usually involve a large number of tests on animals, which could be more ethically or economically justified, especially during the initial stages (Kar & Leszczynski, 2020).

One solution to these problems is rational drug design methods, which employ modern computing resources. Computer models can be an excellent alternative to experiments in specific contexts. Many QSAR/SAR models have been developed, and there are online servers based on them that enable highly accurate predictions of the ADME/T properties of chemical compounds (Borrero, Borrero, Lopez, Pineda, & Castro, 2020). Using in silico experiments during the initial stages of drug development makes it possible to screen out most candidate compounds with unfavourable properties even before starting in vivo and in vitro tests (Kar & Leszczynski, 2020). This approach significantly reduces financial investments, time, and labour costs and saves the lives of millions of laboratory animals (Alqahtani, 2017).

The molecules must possess high biological activity with low toxicity and must also be able to access and concentrate on the therapeutic target in the organism. The traditional approach to considering pharmacokinetics is to break down the various effects that impact access to the target into individual parameters and then evaluate these ADME parameters through dedicated methods (Alqahtani, 2017).

Several methods, including web servers, have been integrated to predict the drug-likeness of molecules. Furthermore, online ADME/Tox resources provide helpful guidelines to filter out compounds that are not likely to be drugs or to extract rational compounds that match the desirable PK properties (Kar & Leszczynski, 2020) (Maltarollo, Gertrudes, Oliveira, & Honorio, 2015).

It is essential to assess the level of toxicity of chemical compounds on humans, animals, plants, or the environment. However, live animal tests can be time-consuming, costly, and have ethical limitations (Kar & Leszczynski, 2020) (Alqahtani, 2017) .As a result, computational methods are deemed reliable for predicting the toxicity of chemicals. In silico toxicology aims to forecast toxicity, prioritize chemicals, provide guidance for toxicity tests, and decrease the likelihood of drug design failures in later stages while enhancing current toxicity assessments (Duran-Iturbedi, Barbara, & Medina-Franco, 2020).

The properties related to absorption, distribution, metabolism, and excretion (ADME) play a crucial role in drug development. A significant percentage, about 40%, of drug failures occur due to issues related to ADME (Alqahtani, 2017). Although preclinical ADME studies have reduced the number of failures caused by pharmacokinetics (PK), drug toxicity remains a problem. Late-stage failures caused by suboptimal ADME and toxicity can lead to a significant loss of time and money (Cunha, et al., 2015). In-silico models are being used to improve ADME prediction, but since the ADME process is complex, decisions must be based on more than one descriptor. Implementing huge data and machine learning can provide a hopeful landscape in predicting ADME and toxicity. Obtaining the in silico ADME/Tox properties of natural products, which are excellent sources of drug candidates, is valuable (Riyadi, et al., 2021).

The development of a new medication depends on the high-quality ADME/Tox properties of a druglike compound and the validation of the target (Li, 2001). Various methods have been integrated into web servers to predict the drug-likeness of molecules. Online ADME/Tox resources are also helpful in providing guidelines for extracting rational compounds that match the desired PK properties or filtering out compounds that are unlikely to be drugs (Alqahtani, 2017) (Domínguez-Villa, Duran-Iturbedi, & Avila-Z arraga, 2021) (Ferreira & Andricopulo, 2019)

Lipinski et al.'s pioneering work established physicochemical ranges for oral drugs by examining orally active compounds known as the Rule-of-five (Daina, Michielin, & Zoete, 2017). This rule delineates the relationship between pharmacokinetic and physicochemical parameters (Domínguez-Villa, Duran-Iturbedi, & Avila-Z arraga, 2021).

### **Lipinski’s rule**

Lipinski's Rule or Rule of 5 outlines specific criteria a substance must meet to be effectively absorbed in the intestine (Zadorozhnii, Kiselev, & Kharchenko, 2022). The substance's molecular weight (Mw) should be less than 500Da, and it should have less than five hydrogen bond donors (NH bond donors) and less than ten hydrogen acceptor bonds (NH bond acceptors). The calculated CLOGP value should also be less than five (or less than 4 for MLOGP). If a substance fails to meet two or more criteria, its absorption in the intestine will be low (Lipinski, Lombardo, Dominy, & Feeney, 1997).

**2.0 MATERIALS AND METHODS**

2.1 Collection, identification authentication and of the plant material

The collection, identification, authentication, plant preparation, fractionation and the selection of the potent fraction have been previously described by (Kankara, Ibrahim, & Onoja, 2024).

2.2 GC-MS full scan analysis:

The nHF was subjected to GC-MS full scan analysis. The separated compounds were identified by comparing their mass spectra with the mass spectral data of the compounds present in the NIST library data base.

2.3 Biological data

Chemsketch and PubChem https://pubchem.ncbi.nlm. nih.gov were used to find 10 bioactive compounds for this investigation [Table 1]. Two-dimensional (2D) images of the selected compound chemical structures of nHF were obtained from PubChem

2.4 Modelling platform

The SwissADME and ProTox II (Prediction of Toxicology of Chemicals) accessible at http://www.swissadme.ch/ and (https://tox new.charite.de/protox\_II/), were used to perform the computational analysis, which included absorption, distribution, metabolism, and excretion (ADME)

2.4.1 ADME Prediction

Accessing http://www.swissadme.ch/ in a web browser displays directly the submission page of SwissADME, where molecules to be estimated for ADME, physical-chemistry, drug-likeness, pharmacokinetics, and therapeutic chemistry properties. Physicochemical, pharmacokinetics and drug likeness properties of the compounds were identified via SwissADME tool. The canonical simplified molecular input line entry system (SMILES) strings of the seven identified compounds from N-hexane fraction of *Catunaregam nilotica* crude methanol extracts were procured from PubChem (https//pubchem.ncbi.nlm.nih.gov/compound). The physicochemical characters of the compounds such as molecular weight (MW), number of hydrogen bond acceptors (nHBA), number of hydrogen bond donors (nHBD) and number of rotational bonds (nRB) were then predicted.

The ADME parameters that include octanolwater partition coefficient lipophilicity (ilogP), solubility, gastrointestinal absorption (GIA), blood brain barrier (BBB), p-glycoprotein (P-gp) substrate, inhibition of isoforms of cytochrome P450 (CYP), and skin permeability (LogKp) were estimated by SwissADME. Lipinski’s rule of five was applied to assess the drug likeness of the compounds. The rule states that drug like compounds ought to have; MW ≤ 500 daltons, nHBA ≤ 10, nHBD ≤ 5 and IlogP ≤ 5. Moreover, compounds are not accepted if they show more than one violation of these set limits (Alqahtani, 2017) (Khare, Chatterjee, Gupta, & Ashish, 2023)

2.4.2 Toxicity Prediction

**ProTox-II** (<http://tox.charite.de/protox_II/>) represents a complimentary online resource that facilitates the prediction of diverse toxicological endpoints associated with chemical substances (Banerjee et al., 2018; Drwal et al., 2014). This platform amalgamates molecular similarity, pharmacophoric data, fragment propensities, and machine-learning algorithms to evaluate toxicity endpoints, including but not limited to acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcome pathways (Tox21), and toxicity targets, thus providing an innovative methodology for toxicity prediction.

### **2.4.2.1Classification Framework**

ProTox-II organizes toxicity predictions into five computational model-based classifications:

1. **Acute toxicity**: Oral toxicity model with five toxicity classes.
2. **Organ toxicity**: Single predictive model.
3. **Toxicological and genotoxicological endpoints**: Includes immunotoxicity, cytotoxicity, mutagenicity, and carcinogenicity (four models).
4. **Toxicological pathways:** Covers 12 models.
5. **Toxicity targets:** Encompasses 15 models.

2.4.2.2 Acute Toxicity & Classification

Oral acute toxicity is quantified as LD50 values (mg/kg body weight), with the accuracy of predictions substantiated through cross-validation processes. Compounds are classified according to the Globally Harmonized System (GHS) for chemical classification and labeling (UN, 2019): Class I: Considered fatal upon ingestion (LD50 ≤ 5 mg/kg), Class II: Considered fatal upon ingestion (5 mg/kg < LD50 ≤ 50 mg/kg), Class III: Categorized as toxic upon ingestion (50 mg/kg < LD50 ≤ 300 mg/kg), Class IV: Deemed harmful upon ingestion (300 mg/kg < LD50 ≤ 2000 mg/kg), Class V: May pose potential harm upon ingestion (2000 mg/kg < LD50 ≤ 5000 mg/kg).

2.4.2.3 Predictive Models

Predictions regarding toxicity endpoints and organ toxicity are derived from both in vitro assays (e.g., Tox21 assays, Ames bacterial mutation assays, HepG2 cytotoxicity assays, immunotoxicity assays) and in vivo investigations (e.g., carcinogenicity, hepatotoxicity). Furthermore, ProTox-II incorporates two target-pathway-based models: Nuclear Receptor Signaling Pathways and Stress Response Pathways.

**3.0 RESULT AND DISCUSSION**

**3.1 GC-MS RESULT**

The GC-MS analysis of fractions of methanolic root bark extract of *Catunaregam nilotica* revealed the presence of several phytochemical constituents that could contribute to the anti-venom effects of root bark of *Catunaregam nilotica* plant. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented in Table 1. N-hexane fraction of *Catunaregam nilotica* contained seven (7) compounds in which 90.2% of the identified compounds were unsaturated fatty acid ranging from C13 to C20, while 9.79% were unsaturated aldehyde hydrocarbon (C16). The first compound identified with less retention time (17.053min) was Eicosanoic acid with 9.45% peak area whereas 11- Dodecenoic acid, methyl ester with 7.94% was the last compound identified with retention time of 25.745min. Oleic acid (66.50%) with the retention time of 20.134min was the major phytochemical identified from n-hexane fraction of *Catunaregam nilotica*.

**Table 1: GC-MS analysis of the n-Hexane fraction of *Catunaregam nilotica root-bark extract***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S/NO** | **Retention Timea** | **Compound Name** | **Molecular formula** | **Molecular weight** | **%Peak area** |
| 1 | 17.053 | Eicosanoic acid | C20H40O2 | 312 | 9.45 |
| 2 | 20.134 | 9-Octadecenoic acid | C18H34O2 | 282 | 66.50 |
| 3 | 21.718 | Hexadecanoic acid1- (hydroxymethyl)-1,2-ethanediyl ester | C35H68O5 | 568 | 1.48 |
| 4 | 22.730 | Octadecenamide | C18H35NO | 281 | 2.03 |
| 5 | 23.135 | 9-Octadecenoic acid-(Z)-,2,3-dihydroxypropyl ester | C21H40O4 | 356 | 2.80 |
| 6 | 23.587 | cis-11-Hexadecenal | C16H30O | 238 | 9.79 |
| 7 | 25.745 | 11- Dodecenoic acid, methyl ester | C13H24O2 | 212 | 7.94 |

**3.1 Physicochemical properties in n-hexane fraction of *catunaregam nilotica***

All identified compounds with the exception of *Hexadecanoic acid-1-(hydroxymethyl)-1,2-ethanediyl ester* had molecular weights within the acceptable range (MW ≤ 500). This suggests that these compounds have the potential to be easily absorbed, diffused and transported into cells.

Drug-like compounds are expected to meet Lipinski's criteria, which include having hydrogen bond acceptors (nHBA) ≤ 10 and hydrogen bond donors (nHBD) ≤ 5. The results showed that all tested compounds adhered to these limits as shown in Table 2. This implies their potential for good absorption and permeability through the gastrointestinal tract when administered.

The compound Hexadecanoic acid-1-(hydroxymethyl)-1,2-ethanediyl ester exhibited the highest number of rotatable bonds (nRB = 19). The compounds with good bioavailability typically have nRB ≤ 15. All other tested compounds fell within this acceptable range indicating favorable permeability and oral bioavailability. However, the high rotatable bond count of Hexadecanoic acid-1-(hydroxymethyl)-1,2-ethanediyl ester (nRB ≥ 15) suggests greater molecular flexibility, which is often associated with poor oral bioavailability.

Lipophilicity is a critical parameter that influences solubility, selectivity, potency, permeability, and promiscuity of lead compounds. The predicted IlogP values for all compounds as shown in Table 2 complied with Lipinski's rule of five (Ro5) (IlogP ≤ 5). Compounds with high lipophilicity (IlogP > 5) are typically associated with rapid metabolic turnover, low solubility, and poor absorption. Furthermore, excessive lipophilicity can increase the likelihood of compounds binding to undesired hydrophobic protein targets, potentially leading to toxic effects within biological systems.

Table 2: The Physiochemical properties and Lipophilicity of n-Hexane fraction of *Catunaregam nilotica*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| S/NO | Compound  Name | Molecular weight | nHBD | nHBA | nRB | ilogP |
| 1 | Eicosanoic acid | 312 | 1 | 2 | 15 | 3.51 |
| 2 | Oleic acid | 282 | 1 | 2 | 15 | 4.27 |
| 3 | Hexadecanoic acid1- (hydroxymethyl)-1,2-ethanediyl ester | 568 | 2 | 1 | 19 | 4.25 |
| 4 | Octadecenamide | 281 | 1 | 1 | 15 | 4.22 |
| 5 | 9-Octadecenoic acid-(Z)-,2,3-dihydroxypropyl ester | 356 | 1 | 2 | 14 | 4.3 |
| 6 | cis-11-Hexadecenal | 238 | 1 | 2 | 15 | 4.01 |
| 7 | 11- Dodecenoic acid, methyl ester | 210 | 0 | 1 | 11 | 3.57 |

Molecular weight ≤ 500 Da; iLogP ≤ 5; hydrogen bond donors (HBD) ≤5; hydrogen bond acceptors (HBA) ≤10

; nRB ≤ 15

**3. Solubility prediction**

Solubility plays a crucial role in drug absorption and distribution. For drug lead compounds to be effectively absorbed, they must exhibit sufficient water solubility to facilitate permeation across cell membranes. The solubility of the identified compounds varied, ranging from soluble to poorly soluble, depending on the applied solubility criteria as shown in Table 3.

Table 3: Solubility predictions of the identified compounds

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Compounds | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| LogS (ESOL) Class | -6.44  (M) | -5.4  (M) | -3.59  (S) | -5  (PS) | -3.59  (M) | -5.73  (M) | -3.51  (S) |
| LogS (Ali) Class | -7.71  (PS) | -8.26  (PS) | -5.14  (M) | -9.97  (PS) | -8.87  (PS) | -8.26  (PS) | -6.51  (M) |
| LogS SILICOSIT Class | -5.61  (M) | -5.39  (M) | -4.39  (M) | -6.91  (PS) | -6.11  (PS) | -5.39  (M) | -4.36  (M) |

Key: S= Soluble; M= Moderate; PS= Poor soluble

**4. The pharmacokinetic parameters**

All compounds except Hexadecanoic acid-1-(hydroxymethyl)-1,2-ethanediyl ester, demonstrated high probability of being absorbed in the gastrointestinal tract. The results further indicated that 30% of the compounds have the potential to cross the blood-brain barrier (BBB). Additionally, almost all compounds were predicted to be non-substrates for P-glycoprotein (P-gp). None of the compounds will inhibit CYP2C19, CYP2D6, or CYP3A4; however, all compounds were shown to inhibit CYP1A2. Furthermore, approximately 50% of the compounds will not inhibit CYP2C9, while 37.5% exhibited inhibitory activity against this enzyme.

Table 4: The pharmacokinetic parameters of the identified compounds

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Compounds | GIA | BBB Permeate | P-gps Substrate | CYP1A2 Inhibitor | CYP2C19 Inhibitor | CYP2C9 Inhibitor | CYP2D6 Inhibitor | CYP3A4 Inhibitor | LogKp  (cm/s) |
| 1 | High | Yes | No | Yes | No | Yes | No | No | -3.05 |
| 2 | High | No | No | Yes | No | Yes | No | No | -2.6 |
| 3 | High | Yes | No | Yes | No | No | No | No | -4 |
| 4 | High | No | No | Yes | No | No | No | No | -1.61 |
| 5 | Low | No | No | Yes | No | No | No | No | -2.19 |
| 6 | High | No | No | Yes | No | Yes | No | No | -2.6 |
| 7 | High | Yes | No | Yes | No | No | No | No | -4 |

The gastrointestinal absorption (GIA) of the identified compounds was predicted and the results are described in Table 4. All the compounds except Hexadecanoic acid1- (hydroxymethyl)-1,2-ethanediyl ester, revealed high probabilities of being absorbed in the gastrointestinal tract. This implies that these compounds have the potential to be absorbed in the gastrointestinal tract upon oral administration. The blood brain barrier (BBB) is the microvascular endothelial cell layer of the brain which separates the brain from the blood. The compounds were evaluated for their ability to cross BBB and the results are shown in Table 4.

The results obtained showed that 30% of the compounds exhibit capability to cross the BBB. The penetration across BBB is only mandatory for compounds targeting the central nervous system (CNS). All Compounds did not show potential to cross BBB; hence this can be an advantage as they have less likelihood to induce adverse effects in the CNS.

P-glycoproteins (P-gp) are membrane transporters of compounds in the intracellular or extracellular directions. Almost all compounds, except compound 2 were estimated to be non substrates for P-gp. This implies that the compounds would not be affected by the efflux action of P-gp, which turns to eliminate compounds from cells, resulting in therapeutic failure because of lower concentrations than expected. Thus, only the efficacy of Hexadecanoic acid1- (hydroxymethyl)-1,2-ethanediyl ester has potential to be resisted in different target sites. Metabolism prediction of lead compounds is one of the main priorities during drug discovery process. The metabolism predictions of the compounds were done against five isoforms of cytochrome P450 (CYP) monooxygenase family namely; CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 and the results are shown in Table 4. All compounds did not inhibit CYP2C19 and CYP3A4 whereas CYP2D6 was only inhibited by compound 7. About 50% of the compounds did not inhibit CYP2C9 while 37.5% did not obstruct CYP1A2. Cytochrome P450 monooxygenase plays a pivotal part in drug metabolization and elimination in biological systems. The non-inhibition action of the identified compounds against these enzymes implies that the compounds have high probabilities of been transformed and consequently be bioavailable upon oral administration.

On the other hand, the inhibition of the CYP isomers by the compounds can lead to poor bioavailability as a result of failure to be metabolised and toxic side effects due to their accumulation. The skin is a selective barrier that allows different compounds to penetrate through at different rates depending on their physicochemical properties. Thus, the skin permeability (LogKp) is a vital parameter for the assessment of compounds that might require transdermal administration. The LogKp of the compounds is presented in Table 3. All the compounds, except Hexadecanoic acid1- (hydroxymethyl)-1,2-ethanediyl ester, are expected to be impermeable as they had the negative LogKp values. This implies that only compound 2 and 4 could be effectively administered through the skin.

**5 Drug-likeness properties and bioavailability of the compounds**

Table 5 shows that most of the compounds have the bioavailable value of 0.55 and 0.85. The 0.55 and 0.85 values imply that the compounds adhere to Lipinski rule of five and have 55 and 85% probabilities of being bioavailable. Only one compound violated Ro5

Table 5: Drug-likeness and bioavailability properties of the compounds

|  |  |  |  |
| --- | --- | --- | --- |
|  | Lipinski’s rule | | |
| Compounds | Satisfactory | Number of violations | Bioavailability |
| 1 | Yes | 1 | 0.55 |
| 2 | No | 2 | 0.85 |
| 3 | Yes | 0 | 0.55 |
| 4 | Yes | 1 | 0.85 |
| 5 | Yes | 1 | 0.85 |
| 6 | Yes | 1 | 0.85 |
| 7 | Yes | 0 | 0.55 |

**6 Toxicological predictions**

According to ProTox Prediction shown in Table 6, the inactive prediction translates compound does not have hepatotoxicity, carcinogenic, neurotoxicity, nephrotoxicity, cardiotoxicity activity. All the identified compounds were found to be safe.

Table 6: Toxicological properties of the identified phytocompounds

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compounds | Hepatotoxicity | Neurotoxicity | Nephrotoxicity | Cardio toxicity | Carcinogenic |
| 1 | Inactive | Inactive | Inactive | Inactive | Inactive |
| 2 | Inactive | Inactive | Inactive | Inactive | Inactive |
| 3 | Inactive | Inactive | Inactive | Inactive | Inactive |
| 4 | Inactive | Inactive | Inactive | Inactive | Inactive |
| 5 | Inactive | Inactive | Inactive | Inactive | Inactive |
| 6 | Inactive | Inactive | Inactive | Inactive | Inactive |
| 7 | Inactive | Inactive | Inactive | Inactive | Inactive |

**Conclusion**

It was observed that majority of the compounds have good physicochemical profiles with several other ADMET properties. The drug-like property predictions showed that most of the compounds, except compound 2 comply with Ro5. All Compounds are safe for use as they did not demonstrate any potential to be carcinogenic on the tested parameters. Furthermore, these predictive results should be validated by in vitro and in vivo toxicological studies.

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