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PHYTOCONSTITUENTS DOCKING: EXPLORING ANTI-INFLAMMATORY TARGETS IN *MUNRONIA PINNATA* AND *ANDROGRAPHIS PANICULATA*

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ABSTRACT

Munronia pinnata (Bin kohomba) and *Andrographis paniculata* (Heen Binkohomba) are two medicinal plants naturally distributed in a wide variety of habitats in Sri Lanka. They have been widely used in traditional medicine. Both plant species are known to have anti-inflammatory properties. However, drug-target interaction patterns of chemical constituents in both plants necessary to optimize targeted drug activity are yet to be studied. Hence, the objective of this investigation was to assess the molecular interaction between phytocompounds from both plants and one of the most prevalent target proteins of anti-inflammatory medications through molecular docking. The chosen phytocompounds were subjected to docking with the cyclo-oxygenase-1 protein target using Auto Dock Vina 1.2.0 to determine their binding affinity values in comparison to the native ligand, Indomethacin. Ligand-based pharmacokinetics, target prediction, and toxicity prediction were assessed using online tools such as SwissADME, Swiss Target Prediction, and pkCSM. The findings demonstrated that the chemical compound with the highest binding affinity in Binkohomba was Campesterol (-8.3 kcal/mol), while in Heen Binkohomba, it was 7-O-Methylwogonin with a binding affinity of -8.8 kcal/mol. Both chemical compounds exhibited superior binding affinity when compared to Indomethacin (-8.1 kcal/mol). Among the two compounds with the highest binding affinity, 7-O-Methylwogonin was projected to possess the most favorable drug-like properties. Based on this study, it is suggested that further exploration of 7-O-Methylwogonin's potential against inflammation should be pursued using advanced computational methods and in vitro experiments.

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Introduction

Inflammation refers to the response of vascular connective tissues towards infections, traumas, injuries, and toxins [1]. When tissues are damaged, the body releases chemical mediators that trigger the response of the immune system. In the event of acute inflammation, the entire process typically lasts for a few hours or days and is accompanied by noticeable symptoms such as redness, warmth, swelling, and pain in the surrounding tissues and joints. On the other hand, chronic inflammation occurs when the inflammatory response remains elevated for an extended period. This prolonged inflammation can lead to white blood cells mistakenly attacking nearby healthy tissues and organs, thereby exerting a detrimental effect on the body. Previous research has demonstrated a correlation between chronic inflammation and various health conditions including heart disease, type 2 diabetes, cancer, arthritis, asthma, as well as bowel diseases like Crohn's disease and ulcerative colitis [2].

Though inflammation is a regular part of the healing process, it is critical to keep it under control to avoid long-term consequences. Eating a variety of foods with anti-inflammatory properties or avoiding inflammatory foods is one way of controlling it [3]. In addition to that, nonsteroidal anti-inflammatory drugs, steroids, and supplements may help to reduce inflammation. Non-steroidal anti-inflammatory drugs (NSAIDs) are substances that inhibit cyclooxygenases by reducing prostaglandin and thromboxane synthesis. In the pharmaceutical industry, cyclo-oxygenase enzymes (COX enzymes) are common targets for anti-inflammatory drugs as they provide relief from the symptoms of inflammation and pain [4]. There

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are two types of cyclo-oxygenase enzymes which are also known as prostaglandin-endoperoxide synthases (PTGS). Among the two types, cyclo-oxygenase-II (COX-2) predominates at sites of inflammation, and cyclo-oxygenase-I (COX-1) is constitutively expressed in the gastrointestinal tract (COX-1). These enzymes are responsible for the formation of prostanoids, including thromboxane and prostaglandins such as prostacyclin, via catalyzing the conversion of arachidonic acid to prostaglandin H2 [5]. COX-1 and COX-2 are isoenzymes that have similar molecular weights, approximately 70 and 72 kDa, respectively. Both proteins have an EGF-like domain (evolutionarily conserved protein domain) at the N-terminus, a short 4-helical membrane anchor, and a core heme-peroxidase catalytic region that shares roughly 65 percent amino acid sequence identity [6]. Many researchers have clarified the link between selective COX-1 inhibition and human pathologies such as cancer, neuroinflammation, cardio protection, fever, and pain. They have also concentrated on the design and development of novel COX-1 inhibitors with excellent selectivity, which may be employed in pharmacological research to learn more about COX-1's involvement in human health and illness. Aspirin, indomethacin, SC-560, FR122047, mofezolac, P6, and TFAP have already been discovered as COX-1 selective inhibitors among the standard NSAIDs [7].

However, long-term use of these medicines can cause serious side effects for users. Long-term NSAID usage can aggravate a variety of chronic conditions, such as heart failure and hypertension, and it can increase the risk of peptic ulcer disease, acute renal failure, and myocardial infarction in adults [8]. The use of medicinal plants is an effective solution to overcome this problem. The presence of important biologically active compounds has made scientists investigate medicinal plants for their uses in treating and management of diseases. Population increase, the high cost of Western medicine, increasing awareness of herbal products, and poverty are also the main reasons behind the increased demand. In addition to that, there is a need to encourage the use of medicinal plants as they are cheaper and more accessible to most of the population. In Sri Lanka, *Munronia pinnata* and its substitute *Andrographis paniculata* have been widely used to treat inflammation.

Munronia pinnata is a threatened, rare species that belongs to the family Meliaceae. It contains several phytochemicals including, phytosterols, fatty acids, sesquiterpenes, and several other types of secondary metabolites. The chemical profiling of *Munronia pinnata* was enabled to unveil the phytochemicals that are responsible for several biological activities. Plant extracts of *Munronia pinnata* have shown an inhibitory activity against 5-lipoxygenase (5-LO) and prostaglandin E2 synthase (mPGES-1) enzymes which implies its' anti-inflammatory activity [9].

Andrographis paniculata is a medicinal plant that belongs to the family Acanthaceae. It is naturally distributed in countries including China, India, Sri Lanka, and other Southeast Asian countries. The plant has been used to cure a variety of diseases in Asia for generations, and it is said to have immunological, antibacterial, anti-inflammatory, antithrombotic, and hepatoprotective effects. The characteristic secondary metabolites such as diterpenoids, flavonoids, and glucosides encountered in the plant have considerably enhanced their therapeutic value [10].

However, in both plants, the chemical constituents that can reduce inflammation are not well understood. In addition to that, the introduction of targets and the mechanism of active compounds of *Munronia pinnata* and *Andrographis paniculata* have not been studied yet. The pharmacological effect of these compounds can be used to optimize targeted drug activity based on the drug-target interaction pattern [11]. Determination of the active compounds that have good binding affinity to the targets is a challenge as a typical drug discovery cycle, from lead identification to clinical trials, is expected to take 14 years and cost around $800 million [11]. Therefore, *in silico* studies with molecular docking can be used to solve this problem. Because the introduction of computer-aided drug design technology could lead to a considerable cost and time reduction in drug design and development.

Molecular docking serves as a crucial technique in the field of molecular biology, enabling the prediction of interactions between proteins (enzymes) and small molecules (ligands). By considering the binding characteristics of the ligand and target, it facilitates the estimation of the three-dimensional arrangement of a complex and generates a range of potential structures. These structures are then ranked utilizing the scoring function within the software [12]. It can be used to execute virtual screening of a large number of compounds, evaluate the findings, and provide structural hypotheses on how ligands suppress the target. Docking scores correlate with the ligand affinity for the target protein. Since the results provide clues about the mechanism of action of the compounds, they can be used to explore molecular interactions of drug candidates with target proteins that bind to each other. ADME studies refer to absorption, distribution, metabolism, and excretion of molecules which can be used to evaluate pharmacokinetic aspects of a drug molecule [13]. It evaluates the rate at which a chemical is absorbed and distributed, the rate and pathways of drug metabolism and excretion, and the plasma attention of a drug over time. Toxicology tests are also performed as part of this procedure. Therefore, this finds about pursuits to discover the right poses of ligands in the binding pocket of a protein and predict the affinity between the ligand and the protein, which presents considerable data associated to computer-based drug sketch inside a shorter duration of time.

Materials and Methods

Sources of Ligands and Receptor Protein

The listing of phytochemicals existing in Munronia pinnata and Andrographis paniculata has been acquired from the earlier published scientific literature **(Table 1)** [8, 14]. A whole of twenty compounds had been chosen for the molecular docking research (Ten from every Andrographis paniculata and Munronia pinnata). Phytochemicals had been chosen for the molecular docking by considering their abundance and bioactivity

Table 1. Chemical constituents present in *Andrographis paniculata* and *Munronia pinnata*

Plant name	Chemical constituents
Andrographis paniculata	Andrographolide 14-deoxy-11,12 didehydroandrographolide Neoandrographolide 14-deoxyandrographolide Andrograpanin 14-deoxy-14,15-dehydroandrographolide Isoandrographolide 3,19-isopropylideneandrographolide 14-acetylandrographolide 7-O-methylwogonin
Munronia pinnata	β-caryophyllene Iso caryophyllene caryophyllene oxide Campesterol Stigmasterol stigmast-4-En-3-one munroniamide neophytadiene ganoderiol F 4,8,12,16-tetramethylheptadecan-4-olide

Preparation of Receptor Protein

The three-dimensional structure of the native ligand Indomethacin (Indomethacin-(S)-alpha-ethyl-ethanol amide) bound to Cyclooxygenase-1 protein was taken from protein data bank (https://www.rcsb.org/). The PDB ID of the target protein was 2OYU. The retrieved protein was first visualized using USCF-Chimera and all intra-molecular hydrogen bonds were removed. Then the protein structure preparation was done using AutoDock tools (version 4.2.6.). In their all-non-standard residues such as HEM, BOG, NAG, and BMA were removed from the initial structure as the ligands found in the pocket proximity do not have any contribution to the binding. Hence those were removed to clear the active site for more efficient calculations. Similarly, water molecules were also deleted to ease the computations and to clear the binding site from possible water molecules that can distort the search as they were not involved in the binding of the substrate or drug. Hydrogens were added to stabilize the ligands as well as the protein itself. All necessary charges and missing atoms were added to the protein before proceeding to the docking experiment to give an optimum condition as macromolecules existing in charged structure with no atom lacking in human physique. Finally, the prepared protein receptor was saved in. pdbqt format and placed in PyRx's workspace folder.

Preparation of Ligands

The 3D structures of *Andrographis paniculata* and *Munronia pinnata* compounds were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/). During this study, the .sdf format of molecules was converted to pdb coordinate using open Babel software (version 2.3.1.) with all hydrogens output format to access AutoDock tools. Then ligand preparation for molecular docking was done using AutoDock tools (version 4.2.6.). Torsions were added to the ligands as they represent the rigid and rotatable pieces. Then the charge is repaired by adding partial gasteiger charges and then forcing AutoDock. The compound is minimized by 10.000 steps to obtain the lowest molecular energy with the most stable conformation and saved in pdbqt format.

Docking Validation

Docking validation used to be carried out for the chosen protein target. Native ligand (Indomethacin) was extracted from protein complex and organized in an equal manner as check ligands. The prepared native ligand was used and then re-docked to its receptor. The grid for docking calculation was centered to cover the protein binding site residues. Resulted grid dimensions were saved in .txt format.

Structure-Based Virtual Screening

All fourteen chemical compounds of the two plants have been docked to the goal protein by the use of AutoDock vina 1.2.0. to achieve their binding affinity values. The same grid size, grid center, and exhaustiveness number were used for the reverse docking simulation as was done in the validation procedure. UCSF Chimera and PYMOL software were used to visualize the docked complexes.

ADME Prediction

ADME studies were used to investigate the pharmacodynamics of the selected chemical constituents which predict how a chemical is processed by a living organism. SWISS-ADME, an online website (http://www.swissadme.ch/) was used to analyze the ligands which had better binding affinities. Canonical SMILES data of the respective ligands were retrieved from PubChem and checked their properties including, ADME parameters, pharmacokinetic properties, druglike nature, and medicinal chemistry friendliness. The SMILES (Simplified Molecular Input Line Entry System) information was entered into

the search bar and was once analyzed.

Target Prediction

The Swiss Target prediction website (http://www.swisstargetprediction.ch/) was used to discover the phenotypical side effects or possible cross-reactivity of the chosen molecules. SMILES records are to be entered into the search bar for the analysis.

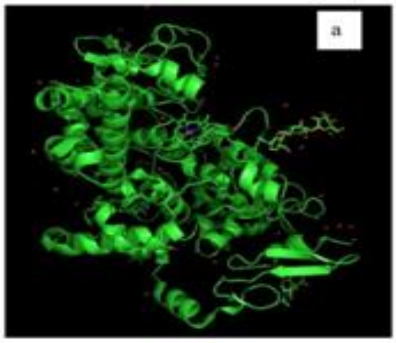
Toxicity Prediction

Toxicity prediction of the selected molecule was done using pkCSM online database (http://biosig.unimelb.edu.au/pkcsm/). SMILES records of the molecules were submitted and toxicology outcomes in the area of AMES toxicity, human most tolerance dose, LD50, LOAEL, Hepatotoxicity, pores and skin toxicity, T. Pyriformis toxicity, and Minnow toxicity were noted.

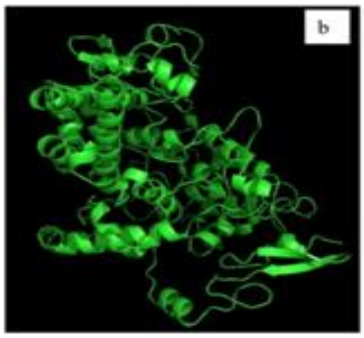
Results and Discussion

Molecular Docking Study

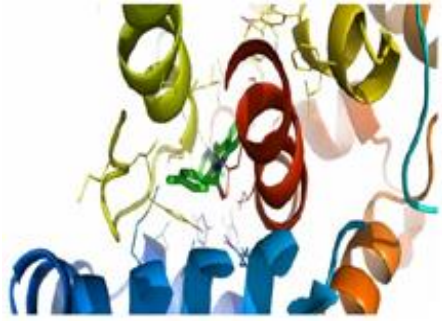
Molecular docking is one of the most applied key tools in structural molecular biology to predict protein-ligand binding activities. It is an invaluable approach to have a better understanding of how small ligands bind to macromolecules. Cyclooxygenase-1(COX-1) **(Figures 1a and 1b)** was selected as the protein receptor because it is one of the most common targets for anti-inflammatory drugs. Since indomethacin inhibits the action of the COX-1 enzyme, it was used as the native ligand **(Figure 1c)**. It inhibits the COX-1 activity either by affecting cyclooxygenase activity or by covalently modifying the enzyme.



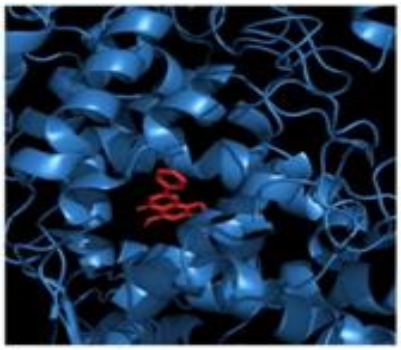
a) Unprepared COX-1 protein



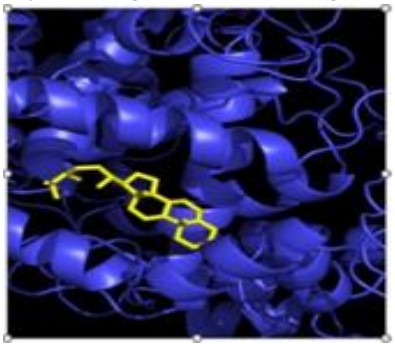
b) Prepared COX-1 protein



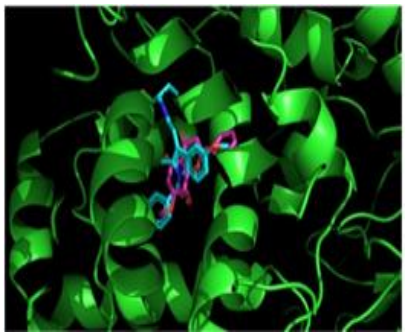
c) co-crystalline ligand Indomethacin (green color)



d) 7-O-Methylwogonin (Red)



e) Campesterol (Yellow)



f) an Indomethacin (blue) and 7-O-methylwogonin (pink)

Figure 1. Protein-ligand binding activities

In order to validate the docking, a native ligand (indomethacin) was extracted from the protein target. The prepared native ligand was then 'redocked' to its receptor. Validation conformed to be valid if the superimposed RMSD of re-docked and

crystallography ligand was less than 2 Å. Validation was confirmed as the superimposed RMSD of re-docked and crystallography ligand 0.64 Å which was less than 2 Å. Auto dock usually requires a pre-calculated grid map, one for each atom type present in the ligand being docked. This helps to compare the selected ligand with the standard ligand. Therefore, grid box settings to determine which ligand bonded space to be docked are shown in **Table 2** and **Figures 1d and 1e**.

Table 2. Grid box dimensions of native ligand Indomethacin

Macromolecule	Ligand	Grid center (A^0)	Grid size (A^0)	Exhaustiveness
Cyclooxygenase-1 PDB ID:2OYU	Indomethacin	CenterX= 20.523 CenterY= 50.052 Center Z=11.247	Size x, y and z=29	8

The aim of further docking of the natural compounds using the same grid dimensions is to predict the structure of a ligand within the constraints of a receptor binding site and to correctly estimate the strength of binding. The result of docking analysis of selected compounds in two plants is described in **Table 3**.

Table 3. Top three Docking results of selected plants derived compounds of each plant against Cyclooxygenase-1 protein

Compound Name	Molecular weight (g/mol)	Binding affinity (Kcal/mol)
Native ligand -indomethacin PubChem CID-3715	**357.8**	**-8.1**
	Munronia pinnata	
Campesterol PubChem CID: 173183	400.7	-8.3
Stigmast-4-en-3-one PubChem CID-5484202	412.7	-7.9
Ganoderiol-F PubChem CID: 471008	454.7	-7.7
	Andrographis paniculata	
7-O-Methylwogonin PubChem CID 188316	298.29	-8.8
14-acetylandrographolide PubChem CID 71589914	392.5	-8.1
Andrograpanin PubChem CID 11666871	318.4	-8.0

Natural products derived from Munronia pinnata and Andrographis paniculata have a wide range of active ingredients that exhibit extensive biological activities. Hence, docking evaluation was carried out for the chosen phytoconstituents towards the COX-1 target protein using the same grid dimensions to consider the efficacy of these compounds in suppressing the activity of the target protein. During the process, it virtually tried to predict a complex by aligning a small ligand molecule internal the binding cavity of the target protein. Then the ensuing docking pose was evaluated through using unique scoring characteristics such as binding affinity. For a ligand to serve as a therapeutic molecule, it must bind to a particular catalytic site with tremendous binding affinity. The superior the ligand binding, the improved the modulating impact, and consequently the ligand with a large binding affinity to COX-1 goal protein can have a higher therapeutic effect. According to the docking scores obtained, it was evident that one phytochemical from *Munronia pinnata* namely Campesterol and another one from *Andrographis paniculata* namely 7-O-Methylwogonin showed higher binding affinities with the target enzyme compared to the native ligand. It proclaims that these compounds have better properties just as the other proposed inhibitors. The rest of the phytochemicals did not show greater interactions with the protein of interest but most of them had considerable binding affinity to the target. The lowest binding energy of -6.9 Kcal/mol was found in Andrographolide and Isoandrographolide. Thus, the *in-silico* docking results, revealed that some of the derived compounds of both plants have great potential against inhibition of COX-1 protein. Therefore, the obtained results can be used to validate their use as a therapeutic agent in the remedy of inflammation. Further, the molecules that had greater binding affinities compared to Indomethacin can be used as lead molecules to produce new drugs that have the most benefits and the least harm.

ADME Prediction

Drug Likeness

Among all compounds, Campesterol and 7-O-Methylwogonin had shown greater binding affinity compared to the native ligand **(Figure 1f)**. Thus, their drug-likeness properties were evaluated to discover the stability of their molecular properties and structural aspects which decide whether these molecules are comparable to recognized drugs. The presence of a range of pharmacophoric features impacts the behavior of molecules internals to a residing organism including, transport properties, toxicity, bioavailability, reactivity, metabolic balance, and many others. SWISS-ADME is an online website that consists of a

set of data that can be used to determine the drug-likeness of a molecule. Drug likeness rules are a set of guidelines that explain the molecular structural requirements of a 'druglike' substance. There are different types of rules including, Lipinski, Veber, Ghose, Egan, and Muegge.

Lipinski's rule of five is commonly used to evaluate the drug-likeness of chemical compounds. It states that an orally energetic drug should not have greater than 5 hydrogen bond donors and ten hydrogen bond acceptors, a molar mass must be lesser than 500 Daltons, water partition coefficient (log p) should no longer exceed 5. All nitrogen and oxygens with at least one hydrogen are considered H-bond acceptors through Lipinski, whilst all nitrogen and oxygens with at least one hydrogen are viewed as H-bond donors. Ghose's rule defines that the molecular weight of small drug-like molecules must be between 160 and 480 Da, the Log p must be between -0.4 and 5.6, and for a total number of atoms, molar refractivity (MR) should be between 40 and 130. If a molecule has 10 rotatable bonds, a topological polar surface area TPSA equal to or less than 140 $Å^2$, and 12 or fewer H-bond donors and acceptors, Veber's model classifies it as a drug. Egan's rule predicts that drug absorption is influenced by mechanisms involving a smaller molecule's membrane permeability. These models represent a molecule as a drug if the log P and TPSA values are 5.88 and 131.6, respectively. The Muegge filter is a pharmacophore point filter that distinguishes between drug and non-drug compounds. These models represent a molecule as a drug if it has a molecular weight of 200 to 600 Da, an octanol/water partition coefficient (X log P) of -2 to 5, a TPSA of 150, several rings of 7, several carbon atoms > 4, some heteroatoms > 1, a number of rotatable bonds of 15, an H-bond acceptor of 10, and an H-bond donor of 5.

Table 4. physiochemical properties of selected ligands and Drug likeness results of selected ligands

Properties	**Campesterol**	**7-O-Methylwogonin**
Molecular weight (g/mol)	400.68	298.29
H-bond acceptors	1	5
H-bond donors	1	1
No of rotatable bonds	5	3
Lipophilicity		
Log Po/w (XLOGP3)	8.80	3.32
Log Po/w (WLOGP)	7.63	3.18
Log Po/w (MLOGP)	6.54	1.01

Molecule	**Druglikeness**				
	Lipinski	**Ghose**	**Veber**	**Egan**	**Muegge**
Campesterol	Yes; 1 violation: MLOGP>4.15	No; 2 violations: WLOGP>5.6, #atoms>70	Yes	No; 1 violation: WLOGP>5.88	No; 2 violations: XLOGP3>5, Heteroatoms<2
7-O-Methylwogonin	Yes;0 violation	Yes	Yes	Yes	Yes

The above results showed that Campesterol violated the drug likeness rules by having undesirable Log P values which could result in poor water solubility. It can hinder oral bioavailability. However, this applies only to oral drugs thus this molecule can be modified and administered through other methods. Because campesterol violated the majority of the drug-likeness score rules, this molecule cannot be recommended as a drug without modification. In the case of 7-O-Methylwogonin, it obeyed Lipinski's Rules with no violations and obeyed drug-likeness score rules such as Ghose, Veber, Egan, and Muegge, which implies its suitability as an oral drug.

In addition to the physiochemical properties mentioned in **Table 4**, the number of heavy atoms, molar refractivity, and topological polar surface area (TPSA) of the two molecules were found. comparing Molecular Characteristics: Campesterol with 29 heavy atoms, 128.42 molar refractivity, and 20.23 $Å^2$ TPSA, versus 7-O-Methylwogonin with 16 heavy atoms, 82.93 molar refractivity, and 68.90 $Å^2$ TPSA. TPSA can be defined as the sum of surfaces of polar atoms in a molecule to analyze drug transport by Multidrug Resistance Protein 1 (MRP1). Scientists suggested that compounds with high TPSA are transported easily. The value obtained for 7-O-Methylwogonin indicates that it has great transport properties.

Pharmacokinetics

A molecule's pharmacokinetic qualities describe how it can function inside the body. It includes information such as the mode and rate of excretion, as well as the duration of the effects. Cytochromes P450 (CYPs) are monooxygenase enzymes that include heme as a cofactor. These proteins oxidize steroids, fatty acids, and xenobiotics in mammals, and are vital for drug clearance as well as hormone production and breakdown. From its 5 main isoforms (CYP1A2, CYP3A4, CYP2C9, CYP2C19, and CYP2D6), cytochrome p450 (CYP) bio transforms greater than 50-90 % of medicinal compounds. P-glycoprotein (P-gp) is broadly distributed throughout the intestinal epithelium, which pumps poisonous metabolites returned into the intestinal lumen as well as from the brain's capillary endothelial cells into the capillaries (For classification, Swiss ADME uses the help vector machine technique (SVM) on datasets of recognized substrates/non-substrates or inhibitors/non-inhibitors. If the

molecule under research is viewed to be a substrate for P-gp and CYP, the ensuing molecule will return "Yes" or "No." respectively.

Table 5. Pharmacokinetic values of the chemical compounds

Ligand	GI Absorption	Pgp Substrate	Inhibitor					Bioavailability Score
			CYP 3A4	CYP1 A2	CYP2 C19	CYP 2C9	CYP 2D6	
Campesterol	Low	No	No	No	No	No	No	0.55
7-O-Methylwogonin	High	No	Yes	Yes	Yes	Yes	Yes	0.55

According to the obtained results, both compounds do not act as p-gp substrates and 7-O- Methylwogonin can interrupt the functions and metabolism of organs. Thus, it is recommended to perform clinical trials to check its' suitability. Based on the results, 7-O-Methylwogonin was predicted to have high gastrointestinal absorption. This indicated that a small amount of these compounds is enough to achieve suitable concentration in plasma and give potential pharmacological effects. Further, both compounds had a 0.55 bioavailability score which implied that it has a 55% probability to reach the systemic circulation. It showed that 7-O-Methylwogonin is a good enduring compound for gastrointestinal absorption through oral ingestion. Medicinal chemistry parameters of 7-O-Methylwogonin were found to be of no pan-assay interference compounds (PAINS) and Brenk alerts. That means this compound will not give false positive results in high-throughput screens. Therefore, it is a revolutionary compound valuable to be tested for biochemical assays The overall results indicate that optimization of this compound may offer the best chance to deliver a 'drug-like' candidate at the end of drug delivery programs.

Target Prediction

Drug goal prediction plays a huge position in the subject of drug discovery as it helps focus on interactions between chemical compounds and the protein goals in the human body. The computational strategies of interplay prediction getting more popular as it is less expensive and less time-consuming compared to wet lab experiments. Swiss Target prediction is an internet site that permits to estimate of the most probably macromolecular ambitions of a small molecule, assumed as bioactive. It was used to analyze the results of 7-O-methylwogonin and the top 25 of the results were displayed in a pie chart **(Figure 2)**.

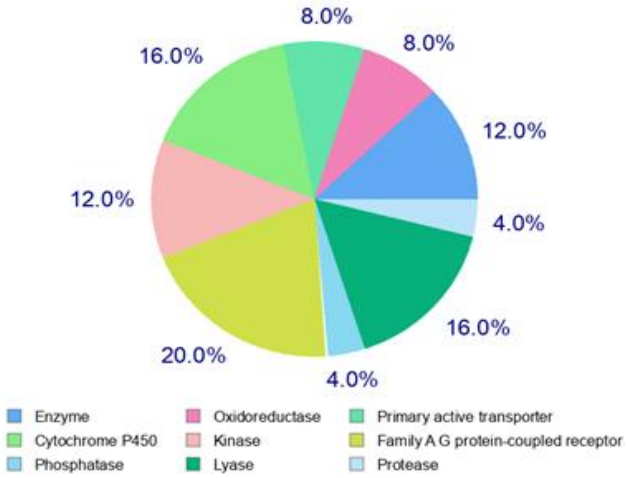


Figure 2. Top 9 macromolecular target groups of 7-O-methywogonin

The possible sites of the target to which the compound may bind were predicted by the software. The pie chart predicts 20% of Family AG protein-coupled receptor, 16% of cytochrome P450 and lyase, 12% of kinase and enzyme, 8% of oxidoreductase and primary active transporters, 4% of phosphatase and protease. According to the literature most of them are considered as 'successful' target families in inflammation [15]. The results showed that the query molecule has an 8% probability of binding oxidoreductase target class which includes COX-1 and COX-2. However, it was not specifically mentioned COX-1 as a target molecule. Therefore, this compound should be further developed using Pka-based modeling-like strategies.

Toxicity Prediction

The toxicity predicted was displayed on the pkCSM website and the outcomes confirmed that the 7-O-methylwogonin no longer has AMES toxicity and as a result no longer purpose any mutations in DNA. It does not inhibit hERG-I and hERG-II. Acute oral rat toxicity (LD50) was discovered to be 2.537 mol/ kg, and Chronic oral rat toxicity (LOAEL) was determined to be 1.238 log mg/kg bw/day. These two measurements can be used when determining the bioactive concentration and the treatment length required. In addition to that it does not cause skin sensitivity and does not produce hepatotoxicity. That means it is not associated with disruption of the normal function of the liver. For a given compound value below, 0.3 log mM is

regarded as high acute minnow toxicity. The value for minnow toxicity was 0.756 log mM and hence not toxic.

Conclusion

Literature searches revealed that the isolated phytocompounds of *Munronia pinnata* and *Andrographis paniculata* have been reported to demonstrate several medicinal properties including, antidiabetic, antimalarial, immunological, antibacterial, anti-inflammatory, antithrombotic and hepatoprotective properties. The use of these plant extracts as a source of therapeutic agents is a key tool to overcome the negative effects that occur due to the long-term usage of synthetic drugs. However, the chemical constituents that are responsible for reducing inflammation and their interactions with protein targets have not been studied yet. Therefore, *an in-silico* study was performed to determine the plant-derived compounds that are effectively bound to the anti-inflammatory target. The study revealed that all the selected compounds possess inhibition properties to a certain extent based on their binding affinities to the target protein. According to the results, 7-O-Methywogonin, Campesterol had more negative binding affinity values against COX-1 target protein compared to the native ligand, Indomethacin. Out of them, 7-O-Methylwogonin was predicted to have the best ADME properties which suggests that, it is an interesting chemical that should be investigated further utilizing sophisticated computational approaches and *in-vitro* tests to see if it has anti-inflammatory properties. Furthermore, the functional groups of these compounds could be altered to reduce their side effects and to increase efficacy during the drug discovery process. Overall, the results can be used to conclude that, both plants consist of phytochemicals that are responsible for anti-inflammatory effects. Thus, they have the potential to be used against inflammatory diseases. However, the efficacy and safety of the compounds need to be further confirmed by clinical trials. It is recommended to carry out these docking studies to evaluate other pharmacological features to validate their therapeutic value. But without knowing the chemical compositions and the concentration differences in different phenotypes of *Munronia pinnata* it is difficult to generalize the results. Therefore, it is recommended to carry out comprehensive research on biochemical variations between different phenotypes of *Munronia pinnata.*

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