Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/5zydra48

EXPLORING COX-1 AND COX-2 INHIBITION POTENTIAL OF *AMBERBOA DIVARICATA* AERIAL PARTS THROUGH IN-SILICO AND *IN-VITRO* STUDIES

Dr. Prasad Mettikolla[1]*, Dr. T. Sunil Kumar Reddy[2], Dr. G. Balammal[2]

[1]*Independent Researcher, A&P Pharmacy INC, 207 E Byron Nelson Blvd, Roanoke, TX-76224, USA.
[2]Rajputana College of Pharmacy, 1 Lake View Street, Coffee Board Layout, Nagavara, Bengaluru - 560024, Karnataka.

***Corresponding Author:** Dr. Prasad Mettikolla
*Email: varam9@gmail.com

ABSTRACT
Acute and chronic inflammatory illnesses continue to be one of the world's most serious public health issues. Although various medications are known to treat inflammatory illnesses, long-term treatment frequently results in severe side effects. This study aimed to identify the COX-1 and COX-2 inhibitory potential of *Amberboa divaricata* through *in-silico* and *in-vitro* studies. The extract was prepared using the ether:petroleum ether (1:2) and dried to obtain the residue which was further used for the assay. The assay was performed using the kit and the ligands (phytoconstituents) were docked against the COX-1 and COX-2 by docking studies. The assay showed an inhibition rate of 61.32% COX-2 and 59.01% (COX-1) at 100 µg/ml. The IC_{50} of the extract residue was 75.10 µg/ml (COX-1) and 70.76 µg/ml (COX-2). The docking studies revealed that only cynaropicrin and desacylcynaropicrin interacted with the active sites of the COX-1 and COX-2 owing to the hydrophilic binding nature of the proteins. Further isolation and preclinical studies is needed to identify the complete potential of the phytoconstituents and their effect on COX-1 and COX-2 targets.

Keywords: anti-inflammatory; *Amberboa divaricata*; docking; inhibition; COX-1; COX-2

1. INTRODUCTION
The inflammatory process is the body's reaction to an injury and infection. It may be triggered by a wide range of harmful substances, including infections, antibodies, and physical traumas [1]. The host's inflammatory response is crucial for interrupting and resolving the infectious process, but it is also frequently responsible for illness signs and symptoms. It entails a complicated set of host reactions, including complement, kinin, and coagulation pathways. Inability to destroy or confine the organism generally leads in further harm owing to inflammation and infection progression [2].
Inflammation can affect persons of all ages. Over-the-counter (OTC) or prescription pain medications such as nonsteroidal anti-inflammatory medicines (NSAID) or corticosteroids are prescribed to moderate or minimise discomfort. Unfortunately, several of these medications have short- or long-term unpleasant side effects such as bleeding, indigestion, heart difficulties, and renal problems. Some, such as opioids, can lead to severe addiction [3].

NSAIDs inhibit the activity of a certain enzyme in your body. These are known as cyclooxygenase enzymes (also called COX enzymes). COX enzymes increase the rate at which your body produces hormone-like compounds known as prostaglandins [4]. Prostaglandins irritate your nerve endings, causing pain. They are also a part of the mechanism that your body uses to regulate its temperature. NSAIDs can reduce pain from illnesses such as arthritis by lowering the number of prostaglandins in your body. They also aid in the reduction of inflammation (swelling), the reduction of fevers, and the prevention of blood clotting. The cyclooxygenase enzyme has two versions, COX-1 and COX-2, which were found in the 1990s [5]. The latter is the one that causes inflammation. COX-1 is recognized to be found in the majority of our bodies' tissues. COX-1 supports the proper lining of the stomach and intestines in the gastrointestinal system, protecting the stomach from digestive fluids [6]. The enzyme is also involved in the function of the kidneys and platelets. COX-2, on the other hand, is predominantly prevalent in inflammatory areas [7]. Both COX-1 and COX-2 create prostaglandins, which contribute to pain, fever, and inflammation. However, because COX-1's major job is to protect the stomach and intestines and contribute to blood clotting, medications that suppress it can have unintended side effects [8, 9, 10]. As a result, there is an urgent need to discover and develop novel anti-inflammatory medications with minimal adverse effects.

Amberboa divaricata, also known locally as Birumdundi, Badaward, or Daaba This plant has traditionally been used as a tonic, aperient, deobstruent, febrifuge, anti-diarrheal, and antiperiodic, as well as for coughs, fever, and general debility. It is both cytotoxic and antibacterial [1]. The seeds have antidote, astringent, and resolvent properties. To alleviate skin irritation, the plant is cooked in water and a bath is taken. In malaria, around 2 g of plant are given and maintained for three days to cure fever [12]. Fresh plant juice combined with black pepper is used to purify the blood. Hence, this study aims to identify the inhibition potential of the aerial parts of *Amberboa divaricata* against COX-1 and COX-2 targets through *in-silico* and *in-vitro* studies.

2. METHODS AND MATERIALS

2.1. Collection and Preparation of extract:

The *Amberboa divaricata* aerial parts were collected, air dried and coarsely powdered. The powder were extracted with Ether and Petroleum ether (1:2) and concentrated under reduced pressure to form a semisolid mass. The residue was preserved in an airtight container for future use [13].

2.2. Drug-likeness prediction:

The drug likeness properties of the ligands were evaluated using the qikprop tool in the Schrodinger suite [14].

2.3. Molecular Docking:

2.3.1. Ligand Preparation:

Ligands included betulinic acid, stigmasterol, lupeol, aguerin-B, cynaropicrin, desacylcynaropicrin, lupeol acetate, β-sitosterol, and β-sitosterol-D-glucoside. The ligands were then prepared for docking with the Ligprep tool, and the OPLS4 field force was used since it has been shown to be more accurate [15]. Following ligand pre-processing, the final docking ligands were selected based on state penalty ratings. The best ligands were chosen based on the penalty scores.

2.3.2. Protein Preparation:

Two different target proteins were chosen namely 5KIR [16] and 3KK6 [17]. The proteins were preprocessed by adding the missing chains, hydrogens and atoms. Then the protein was optimized and minimized before performing the docking. [18].

2.3.3. Docking:

The receptor grid was created using the active site interactions of the protein's existing ligands. The studied grid was chosen for each protein and docking was performed. Docking was carried done

with standard precision, with the output set to 15 poses per ligand. Finally, the poses were examined in order to find the interactions.

2.4. COX-1 & COX-2 inhibition activity:

The capacity of *Amberboa divaricata* to inhibit COX-1 and COX-2 isoenzymes in vitro was determined using an assay kit according to the manufacturer's instructions [19]. The experiment was carried out with reaction buffer solutions of TRIS-HCl buffer (0.1 M-pH 8.0) including cofactors hematin (1.0 mM), phenol (2.0 mM), and EDTA (5 mM). The test materials were diluted in DMSO and examined in final volumes of 1 mL at concentrations of (10, 20, 40, 60, 80, 100 µg/ml), reference substance (1 mM), or vehicle (DMSO, 1.0 percent) [20]. A unit of ovine pure COX-1 or COX-2 was suspended in the reaction medium and preincubated at room temperature for 5 minutes. The reaction was then started using arachidonic acid (5.0 mM) and incubated at 37°C for 20 minutes. The COX reaction was halted by adding 50 mL of 1 M HCl. Positive controls for the COX-1 and COX-2 assays were indomethacin and NS-398, respectively [21]. The absorbance was determined using an ELISA reader. Percentage (%) inhibition was estimated by comparing *Amberboa divaricata* to a blank and using the following equation:

% inhibition = $PGE2_{vehicle} - PGE2_{extract}$ X 100 / $PGE2_{vehicle}$

3. RESULTS AND DISCUSSION

3.1. Drug-likeness prediction:

The drug-likeness property of the ligands used for docking were analyzed using the qikprop tools in the suite. The molecular weight, no. of h-bond donor, acceptor and o/w partition coefficient were determined and tabulated (Table1). Few ligands violated the drug-likeness properties but these ligands considered for the *in-silico* studies in order to determine any interaction with the proteins.

Table 1. Drug-likeness properties of ligands

S.no	Ligands	Molecular weight	H –bond acceptor	H-bond donor	O/W partition coefficient
1	cyanaropicrin	426.724	1.7	1	7.087
2	Lupeol Acetate	468.762	2	0	8.008
3	Desacylcynaropicrin	262.305	6.4	2	1.1
4	Stigmasterol	412.698	1.7	1	7.737
5	Beta-Sitosterol	414.713	1.7	1	7.622
6	Aguerin B	330.38	6.7	1	2.356
7	Cynaropicrin	346.379	7.4	1	1.88
8	b-sitosterol-d-glucoside	576.855	10.2	4	5.173
9	Betulinic Acid	456.707	3.7	2	6.24

3.2. Molecular Docking

3.2.1. 5KIR

The ligands were docked against the COX-2 protein which already had rofecoxib bound to the active site. The same site was chosen for the ligands to interact. It was found that only cynaropicrin and desacylcynaropicrin showed interaction with the active site of the protein (Table 2). The active site was analysed to identify the residues and loop responsible for the conformational change and inhibition. It was found that the loop attracts only hydrophilic interaction and opposes the hydrophobic interaction. Residues such as HIS90 and ARG513 were responsible for the conformational change and only allows hydrophilic interaction. Comparing the o/w partition coefficient of the ligands it is evident that only cynaropicrin and desacylcynaropicrin show hydrophilcity with low value when compared to the other ligands. Hence no interaction of

hydrophobic molecules were found. The desacylcynaropicrin showed a high docking score and interacted with the residues HIS90 and LEU353 (Figure 1). Cynaropicrin did not show any interaction with the residues but were found to fit inside the pocket of the active site.

Table 2. Docking scores of ligands against 5KIR

S.No	Ligands	Docking score	Glide score	Glide e-model score
1	desacylcynaropicrin	-8.099	-8.099	-24.810
2	cynaropicrin	-6.834	-6.834	-33.098

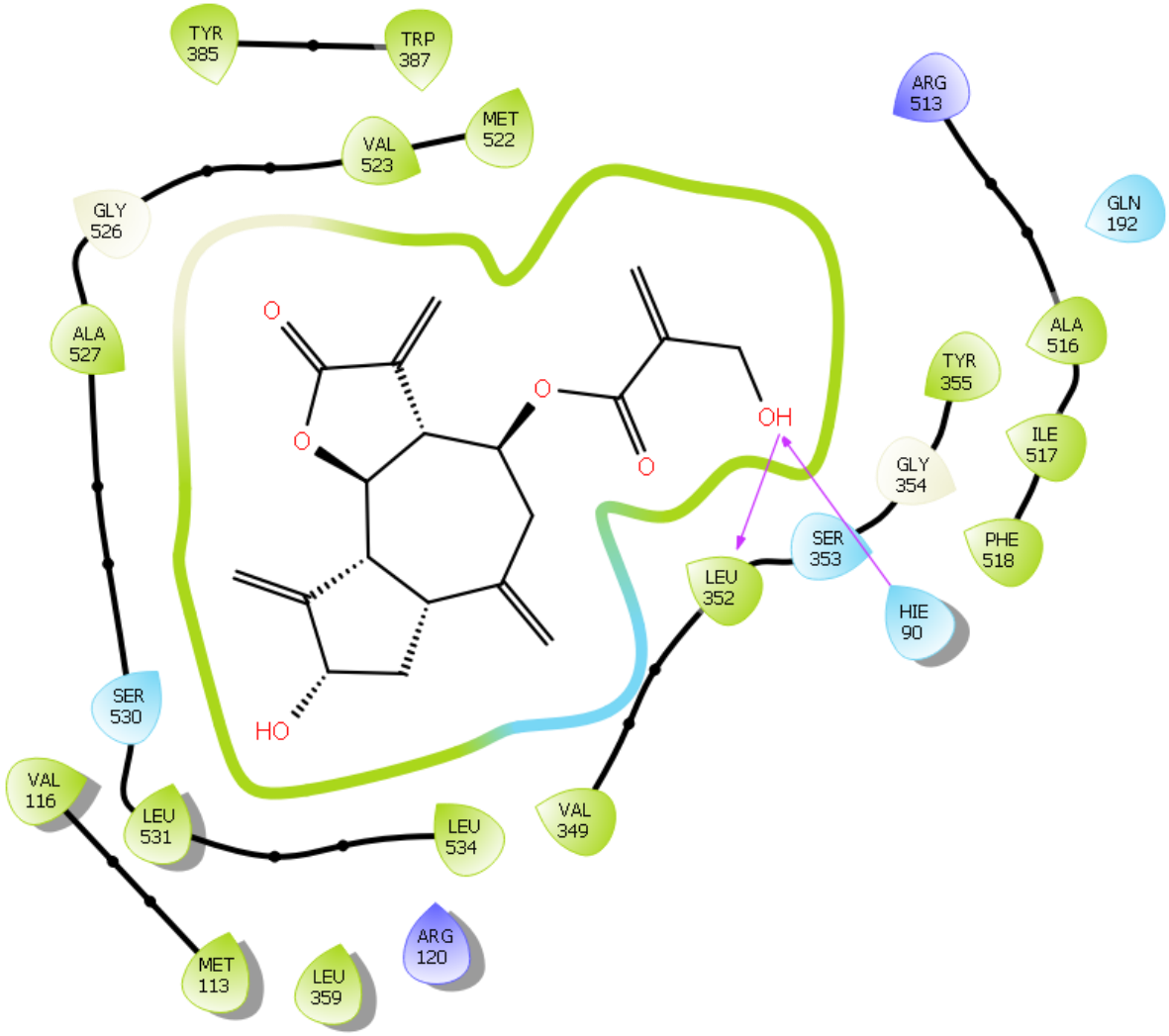

Figure 1. Interaction of desacylcynaropicrin with 5KIR

3.2.2. 3KK6

Similarly the ligands were also targeted against the COX-1 and the results were tabulated (Table 3). Only cynaropicrin and desacylcynaropicrin were found to interact with the active site of the protein owing to the nature of the pocket. Similar to the COX-2 active site, COX-1 also has residues that cause the conformational change. Residues 513-520 are responsible for the changes as the protein contains celecoxib. Interaction of cynaropicrin with these residues may cause the conformational change and inhibit (Figure 2).

Table 3. Docking scores of ligands against 3KK6

S.No	Ligands	Docking score	Glide score	Glide e-model score
1	cynaropicrin	-8.561	-8.562	-71.394
2	Desacylcynaropicrin	-7.730	-6.834	-43.685

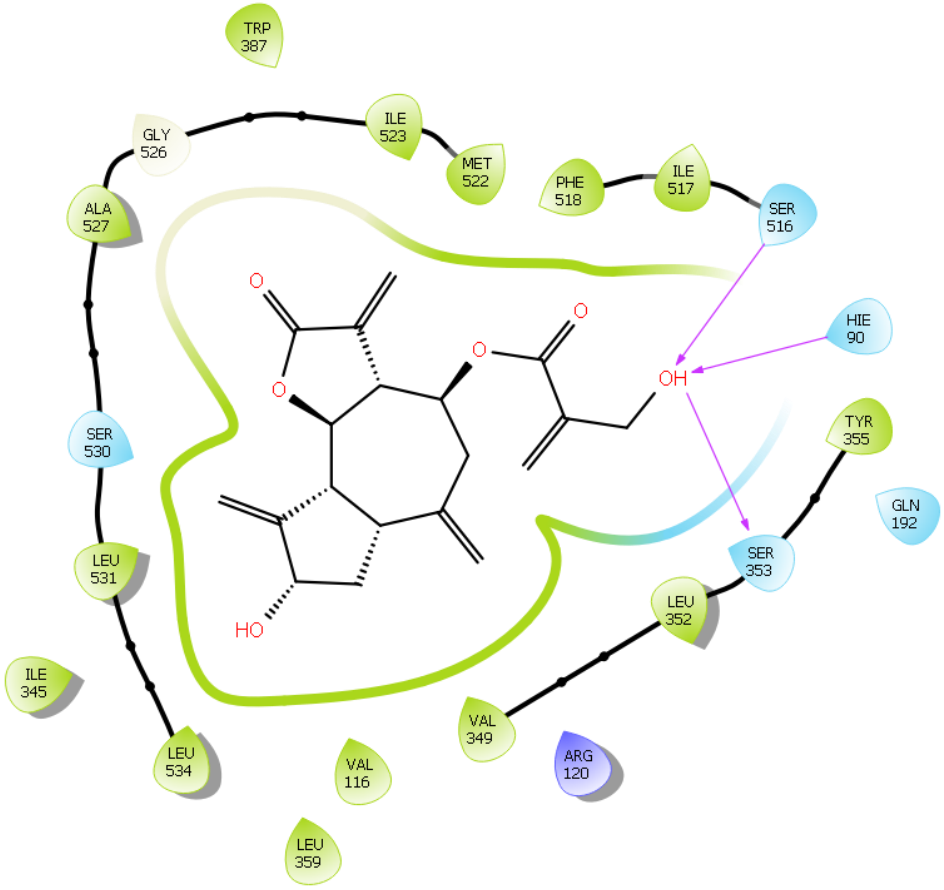

Figure 2. Interaction of cynaropicrin with 3KK6

3.3. COX-1 & COX-2 inhibition:

The extract residue was subjected to the assay (absorbance – 410nm) and it was that the phytoconstituents inhibited the COX-2 with 61.32% at 100 µg/ml and COX-1 with 59.01% at 100 µg/ml in a dose dependent manner. The IC_{50} of the extract residue was 75.10 µg/ml (COX-1) and 70.76 µg/ml (COX-2) (Figure 3 & 4).

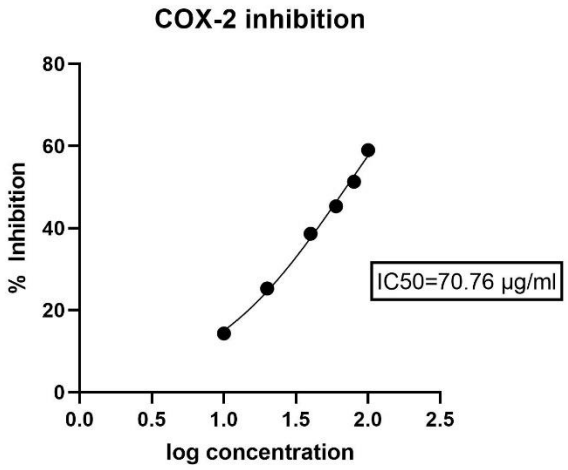

Figure 3. COX-2 inhibition assay

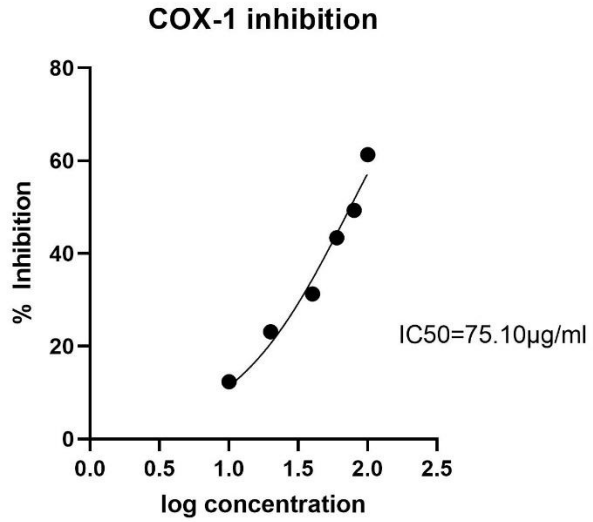

Figure 4. COX-1 inhibition assay

4. CONCLUSION

The aerial part of *Amberboa divaricata* was extracted with ether and petroleum ether (1:2). Also, the phytoconstituents reported were docked against COX-1 and COX-2 by molecular docking studies. It was found that only 2 compound showed interaction with the proteins targets. Similarly the COX-1 and COX-2 assay was performed using the kit. The extract showed a minimal inhibition rate. The inhibition effect of the extract on COX-1 and COX-2 may either be due to the cynaropicrin and desacylcynaropicrin or the synergistic effect of the phytoconstituents present in the extract. Further isolation of the phytoconstituents is required to understand the complete effect of the plant.

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