Pharmacophore Modelling Analysis of Burdock Root Extract and Vanillin Derivatives as Anti-Inflammatory Remedy

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ABSTRACT Pharmacophore modelling is an important aspect towards modern medicinal chemistry in drug discoveries and computer aided drug design to ease the understanding between the receptor-ligand interactions. Burdock (*Arctium lappa*), a well-known Traditional Chinese Medicine used in various natural therapeutics was chosen due to its anti-inflammatory characteristics of its constituent (arctiin and arctigenin) which meant to inhibit the metabolism of xanthine oxidoreductase. In this study, virtual screening comparison of burdock root constituent and vanillin derivatives were done *via* structure-based and ligand-based pharmacophore modelling towards the inhibition of xanthine oxidoreductase using Ligand Scout 4.1 software. From pharmacophore modelling analysis burdock root constituents (arctiin and actigenin) are the best anti-inflammatory compared to vanillin derivatives and clinical applied remedy.

KEYWORDS: Structure Based Drug Design, Ligand Based Drug Design, Gout, Burdock Root and Vanillin Derivatives.

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TRANSACTIONS ON SCIENCE AND TECHNOLOGY

INTRODUCTION

Pharmacophore is defined as 3D arrangement of chemical groups common to active molecules and essential for their biological activities. Pharmacophore modelling is an important tool in drug discovery and in modern medicinal chemistry which was introduced by Ehrlich in 1909 to ease the understanding between the receptor-ligand interactions (Yang, 2010). Structure-based pharmacophore modelling is a strategy in the presences of a target macromolecule to observe the interaction of proposed drug with the active site. Ligand-based drug pharmacophore modelling is a strategy to discover potential drug by generating a pharmacophore model using drug that are being used to treat a diseases or infection without the involvement of a macromolecule.

Gout has become more ordinary among elderly people at a point of their lives due to the longer life expectation and change in diet. Gout is an inflammatory joint disease caused by tiny grit-like crystal in the joint due to high level of uric acid in blood which leads to swelling and pain (gout attack) (Kenny, 2014). Xanthine oxidase is the major enzyme in production of uric acid and also reported to produce superoxide radical and hydrogen peroxide which leads to oxidative stress, inflammation, cancer, aging and many more (Cos *et al.*, 1998). The following chemical reactions are catalysed by xanthine oxidase towards formation of uric acid (Birkett *et al.*, 1997).

$$\text{Hypoxanthine} + H_2O + O_2 \longleftrightarrow \text{Xanthine} + H_2O_2$$
$$\text{Xanthine} + H_2O + O_2 \longleftrightarrow \text{Uric Acid} + H_2O_2$$

Inhibition of this enzyme leads to the reduction of uric acid and prevent gout attacks (Pacher *et al.*, 2006). Allopurinol, febuxostat and other xanthine oxidase inhibitors are consumed, however side effects are tangible and cannot ingest along with other medication such as medication for heart attacks and etc.

Burdock (*Arctium lappa)*, has been a popular Traditional Chinese Medicine to treat arthritis, cancer, liver tonic, diaphoretic and diuretic, blood purifier, laxative, antipyretic and antimicrobial (Chan *et al*., 2011). Based on the research done by Chan and friends (2011), burdock inhibits inducible nitric oxide synthase (iNOS) expression, nitric oxide (NO) production, proinflammatory cytokine expression, nuclear factor-κβ (NF-κβ), antioxidant enzyme activation, and free radical scavenging (Lin *et al*., 2002). One of the important pharmacological effects this plant posesess is anti-inflammatory (Lin *et al*., 2002).

Vanillin is the major component of natural vanilla, one of the most widely used as flavouring materials worldwide and used for pharmaceutical approach for drug development. Vanillin show the properties such as antifoaming agents and antimicrobial agent (Hocking, 1997), antimutagenic effect and antioxidant is used as food preservatives (Davidson & Naidu, 2000). It is reported that vanillin has the characteristics of anti-inflammatory by Srikanth and friends (2013) and Niazi and friends (2014). Vanillin derivatives proposed using Schiff base reaction contains azomethine group which is known in the biological applications especially towards anti-inflammatory.

Due to the similar effect of anti-inflammatory of vanillin and burdock root constituent, this study is important to screen three clinically approved gout remedy, burdock root constituents and vanillin derivatives using pharmacophore modelling.

METHODOLOGY

The *In-Silico* screening evaluation which is pharmacophore model generation and validation were done with the aid of LigandScout 4.1. The training sets which are the available and utilized drug to treat gout (allupurinol, febuxostat and cholchine) and test set (vanillin derivatives and burdock root constituent) were first drawn in Chem Draw and saved in the mol. format then introduced into the tool to minimize the energy level. The major chemical features which can be obtained are Hydrogen Bond Donor (HBD), Hydrogen Bond Acceptor (HBA), Hydrophobic (Hy) and Aromatic (Ar).

Pharmacophore Model Generation

Pharmacophore model was generated in two approaches; which were ligand based and structure based. In ligand-based approach, training sets were aligned and merged in alignment perspective feature to generate pharmacophore model. While, in structure-based pharmacophore model generation, a 3D "Xanthine Oxidoreductase" molecule was retrieved and downloaded from Protein Data Bank (PDB) with the entry code of 3EUB into LigandScout 4.1. The retrieved enzyme consists of several binding sites but only one of the active site bonds with a substrate to generate the pharmacophore model. The exclusion volume was added to the pharmacophore model before the validation process.

Pharmacophore Validation

Pharmacophore validation is where the training sets are aligned with the pharmacophore model to obtain superimposed model. Validation procedure is based on the Fischer's randomization test, which checks whether a strong correlation exists between the chemical structures and the biological activity. The compound is used to quantify the validity, and finally candidate molecules are mapped onto the model and their activities predicted.

In ligand based drug design, all of the test sets was aligned with the generated pharmacophore model. These processes were done in ligand base perspective where the ligand and pharmacophore

was superimposed. In structure based drug design, the training and test sets were aligned into the pharmacophore model which contain exclusive sphere. These processes were done one at a time in alignment perspective where the chemical structure and features were aligned. Once the sets were successfully aligned with the pharmacophore model, it was introduced to structure base perspective to be docked and the interactions between set and protein were virtually viewed.

RESULT AND DISCUSSION

Ligand Based Pharmacophore Modeling

(a) Pharmacophore Model Generation

From the training sets a pharmachopore model was successfully generated with the chemical features of 3 HBA. From the generation of the model (Figure 1), we can determine that HBA is a major chemical feature towards gout treatment. In allopurinol 2 HBA were generated at the nitrogen of C=N and 1 HBA at oxygen of C=O, whereas for febuxostat 1HBA atnitrogen of C=N, 1HBA at oxygen of O-H and 1HBA at oxygen of C=O and finally for colchicine 3HBA at the oygen of the ether group. Further a fit value for the training sets was also obtained as shown in Table 1.

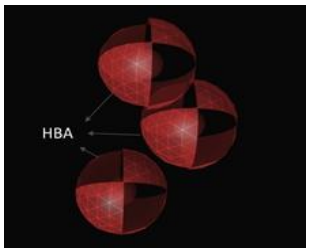

Figure 1. Generated pharmacophore model.

Table 1. The chemical features and pharmacophore fit values of the training sets.

Name	Types	Matching Features	Pharmacophore-Fit
Febuxostat	Training		37.91
Colchicine	Training		38.02
Allopurinol	Training		37.80

From the table, colchicine has the highest fit value of 38.02 with the pharmacophore model followed by febuxostat, 37.91 and allopurinol with the fit value of 37.80 was obtained.

(b) Pharmacophore Validation

Once the pharmacophore model was generated and the chemical features and fit value for the training sets were obtained, the test sets was superimposed with the pharmacophore model. Figure 2, shows the 2D of the selected test sets superimposed with pharmacophore model and fit values. Based on the figure, **arctiin** and **arctigenin** possesses a higher fit value compared to the training sets in table 1 with the fit value of 38.52 and 38.46 respectively. These shows that arctiin and arctigenin are more suitable to be used compared to training sets (alluporinol, febuxostat and colchicine). Whereas for the vanillin derivatives possesses less fit value compared to arctiin, arctigenin and training sets. In test sets of the vanillin derivatives, **C14** has the highest value with 37.42 whereas **C4** has the least fit value of 35.36. **C5** does not possesses and fit value due to lack of chemical features and unable to be superimposed with the pharmacophore model.

The slight differences in the fit value are due to the interaction of the chemical features with the functional group of each training sets and test sets. For the 1st HBA the trend of interaction is strongly through the ether group found in **arctiin, actigenin** and **colchicine**, followed by interaction through the carbonyl group found in **febuxostat, allopurinol** and **C14** and finally interacts through

hydroxyl group found in **C13, 10, 20, 15, 12** and **4** respectively per fit values. The 2nd HBA, strongly interacts through hydroxyl group found in arctiin, followed by interaction through ether group found in arctigenin and colchicine and finally interacts through imine group found in febuxostat, allopurinol and the vanillin derivatives. 3rd HBA, strongly interacts through hydroxyl group found in febuxostat, followed by interaction through the imine group found in allopurinol and C14 and finally interacts through ether group found in arctiin, arctigenin, colchicine and other vanillin derivatives. However, the 3rd HBA of arctiin, arctigenin and colchicine interacts with ether group and have a higher fit value due to the influences of the 1st HBA and 2nd HBA.

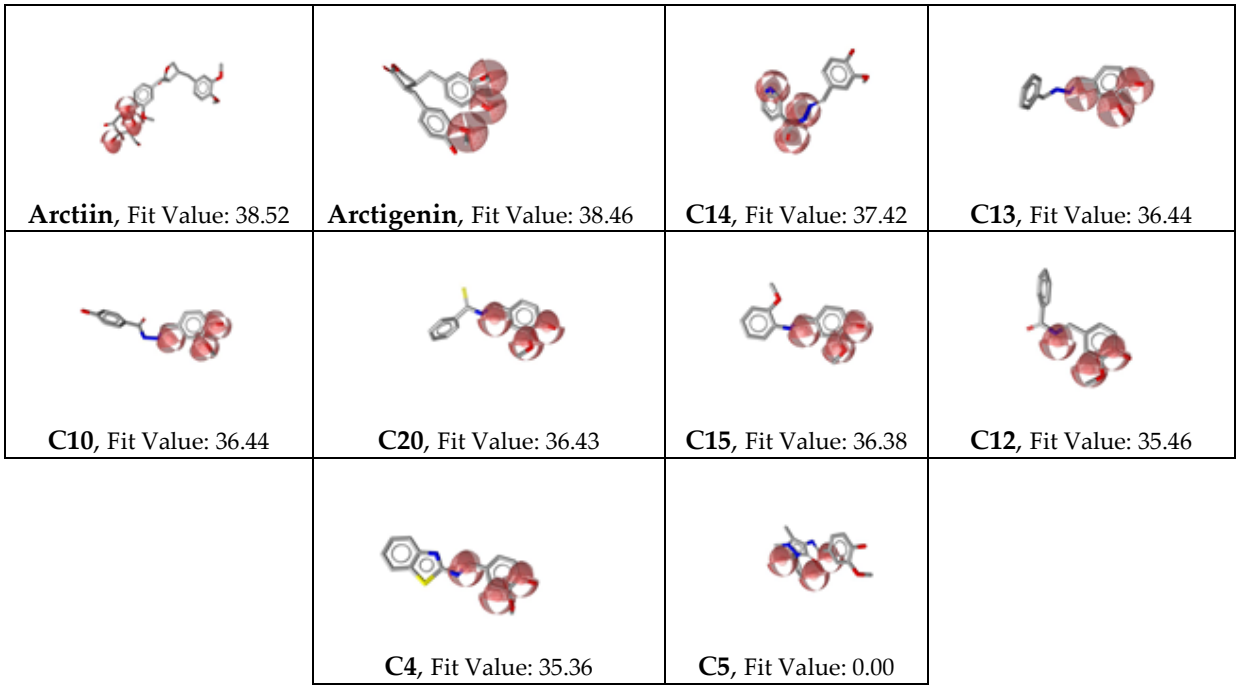

Figure 2. 2D chemical structure interaction with chemical features with fit values.

Structure Based Pharmcophore Modeling

A) Pharmacophore Model Generation

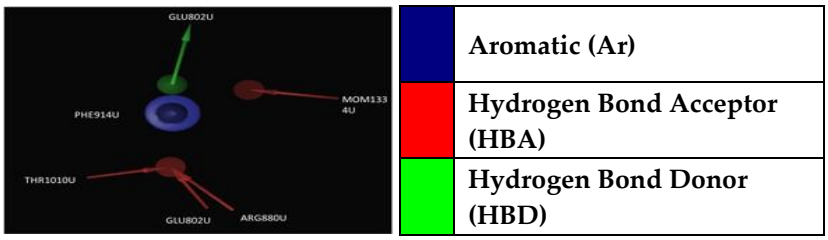

Figure 3. Generated pharmacophore model with chemical features and interaction with amino acid

Figure above show the pharmacophore model generated from selected active site of Xanthine Oxidase containing xanthine as substrate. The pharmacophore model consists of 1 Ar, 1 HBD and 4 HBA. The HBD interacts with the gluatmic acid, Ar interacts with phenylalanine and HBA interacts with the exclusive volume, arginine, glutamic acid and threonine.

B) Pharmacophore Validation

The training sets were introduced into the structure based perspective to observe the docking process into the active site. Figure 4 shows the docked training sets and 2D interaction of the training sets in the active site.

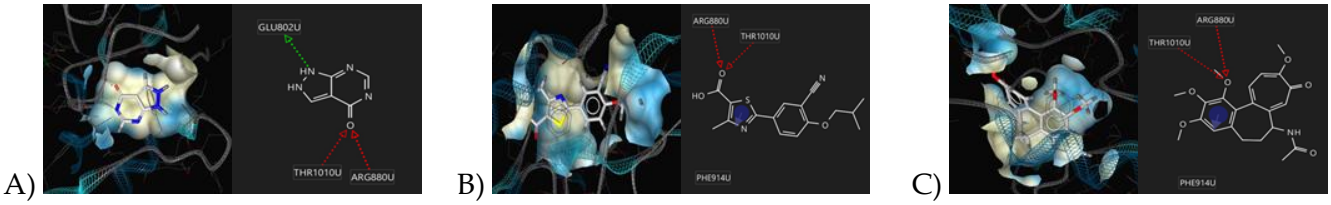

Figure 4. Training sets A) Allopurinol, B) Febuxostat and C) Colchicine in the binding pocket of active site and 2D interaction of training sets.

Based on Figure 5, allopurinol has two interaction which is 1HBD interacts with glutamic acid and 2HBA interact with threonine and arginine whereas for febuxostat and colchicine have two interaction which is 1 Ar interacts with phenylalanine and 2 HBA interacts with threonine and arginine. Figure 5 shows the docking of test sets into the binding pocket and 2D interaction of the test sets per figure 2.

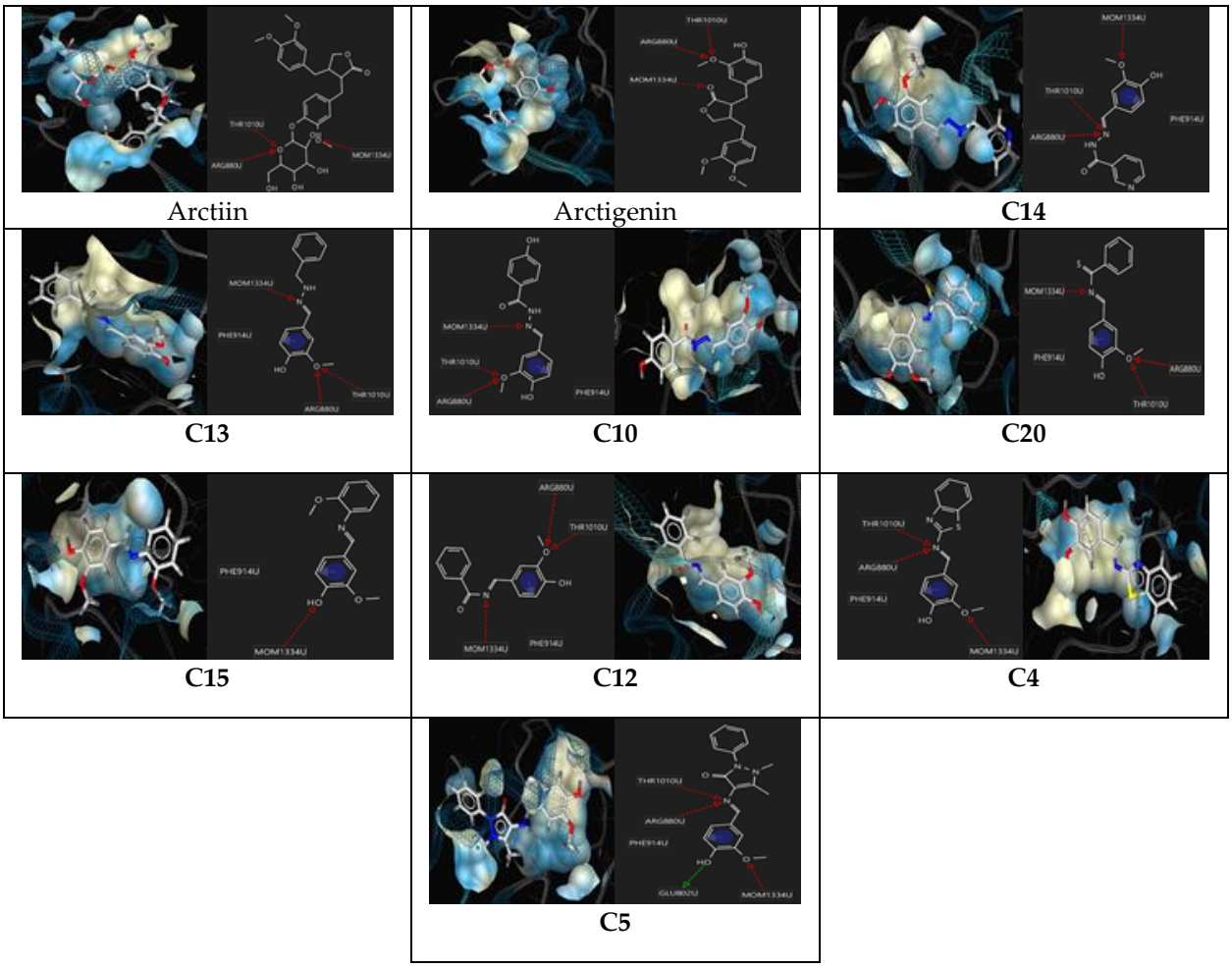

Figure 5. Docking of test sets in Binding pocket and 2D interaction of test sets with amino acid

Based on the docking analysis, all of the test set were able to bind into the active site of Xanthine Oxidase. Burdock constituent (arctiin and arctigenin) has few interaction with the binding site which consist of 3 HBA interaction with threonine, arginine and exclusive volume. The 3 HBA is an important chemical feature in possessing anti-inflammatory properties. **C5** from the vanillin derivatives has the most interaction compared to the other vanillin derivatives. The other vanillin derivatives consists of 3 HBA interacts with threonine, arginine and exclusive volume and 1 Ar interacts with the phenylalanine except for **C15** which only have 1 HBA which interacts with exclusive volume and 1 Ar interacts with phenylalanine.

CONCLUSION

From ligand-based pharmacophore modelling, HBA is important to observe for a test set to possess the properties of anti-inflammation. In structure-based pharmacophore modelling, 3 HBA, 1Ar and 1 HBD chemical features which interacts with glutamic acid, threonine, arginine, exclusive volume and phenylalanine are important to bind to the binding pocket of the macromolecule.

As a conclusion, burdock root constituents (arctiin and arctigenin) are suitable to be used to treat gout based on pharmacophore modelling, whereas for vanillin derivatives C14 is suitable to be used to treat gout per ligand based pharmacophore modelling and C5 is suitable to be used to treat gout according to structure based pharmacophore modelling.

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