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FACULTY OF SCIENCE EDUCATION

DEPARTMENT OF CHEMISTRY

In silico identification of potential antimalarial mechanism of components of bidens pilosa

By

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This Final Year Project Proposal is submitted to the Department of chemistry in partial fulfillment of the requirement for the award of the degree of bachelor in education of Busitema University.

JULY 2024

DECLARATION

ILUMBUKU LABAN declare that the work has been collected by myself. In addition, the work has not been submitted for any other degree or professional qualification. I confirm that the work is my own. My contribution and those of other authors to this work have been explicitly indicated below. Appropriate credit has been granted to the origin of some work in the thesis where reference has been made to the creation of others.

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DR. ANDIMA MOSES

Signature..... Date.....13/8/2024.....

DEDICATION.

With much love, Enthusiasm and joy, I do dedicate my work to all my mentors especially **DR. EGOR MOSES**, a unique feeling of gratitude of my beloved parents**, MALONGO JOSEPHINE** and **LUMBUKU PAUL** for there un ending words of encouragement, motivation and push for another step forward in my career. My family has been there for me and I am glad about that as it rejuvenates me.

I also with a Godly heart dedicate this work to my supervisor, **DR. ANDIMA MOSES**, my brother **NALYANGA BRIAN** who has supported and guided me throughout the entire process right from the start to the accomplishment of this my research. I will always appreciate all his efforts that have helped me to develop my thinking skills, technological skills and creativity.

I dedicate this work and much appreciation to my best friend **KITONGO LYDIA MUKWANA** & my sister **KAKAYI DAMALI** for being there for me in times of need. You two have been my best and have created a strong bond with me.

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I would like to appreciate the work of my supervisor **DR. ANDIMA MOSES** who diligently guided me during my research. May the Almighty reward him abundantly?

Special thanks goes to my father for her love and support in form of school fees for my education.

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I also wish to thank my teachers for their advice, knowledge and guidance towards my education. May God bless them.

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ABSTRACT

Bidens pilosa is an annual species of herbaceous flowering plant in the daisy family Asteraceae and is a small shrub that originated from South America and spread around the world. It has small yellow and white flowers. It has been proven to exhibit medicinal properties including the antimalarial property. However, the mechanism by which bidens pilosa treats malaria remains unclear.

Malaria is a serious and sometimes a fatal mosqito-borne disease. It is the leading cause of morbidity and mortality in many developing countries in particular Uganda where young children and infants, pregnant mothers and their unborn babies are the most affected groups.

In this research an in silico study of the molecular mechanism of action of bioactive phytochemicals from bidens pilosa in the treatment of malaria through network pharmacology and molecular docking was carried out.

This research was conducted to assess the molecular mechanism of bidens pilosa bioactive phytochemicals towards the treatment of malaria through network pharmacology and molecular docking.

The methods used were ;

Bioactive phytochemicals compounds of bidens pilosa were obtained from publicity literatures. Chemical repository server called PubChem website was used to obtain the canonical SMILES (Simplified Molecular Input Line Entry System) of the bioactive compounds.

Network pharmacology. This involved target prediction of the ligands SwissTarget predicton and TargetNet databases, identifying malaria-related genes and intersection genes using DisGenet, Genecards, Online Mendelian Inheritance in Man (OMIM) and Interactive Venn website to screen the intersection genes

Protein-Protein Interaction network connections and visualizations using STRING databae and Cytoscape after which the core targets targets were determined and finally docking between the receptor-protein and ligand was carried out in the Molecular Operating Environment (MOE)

window. The binding energy of the interaction the target and the receptor-rotein was calculated and measured in kcal/Mol

The ligands screened fom different databases were Ferullic acid, 7-Phenyl-hepta-2,4,6-triyn-2-ol, Gallic acid, Trideca-1,11-diene-3,5,7,9-tetrayne, Lauric acid, Dimethoxyphenol, salicylic acid, 2-methyl-5-propan-2-ylcyclohexa-1,3-diene ,1,6-dimethyl-4-propan-2-yl-3,4,4a,7,8,8a-hexahydro-2H-naphthalen-1-ol, 3,7-dimethylocta-1,6-dien-3-ol, 4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-ol which were docked with each of the following intersection genes (malaria-related genes); IL2, PTGS2, MMP9, TLR4, CASP3 HMOX1, ALB, TNF and CXCL8

From the results of docking it was observed that PTGS2, was a core target of Ferullic acid, 7-Phenyl-hepta-2,4,6-triyn-2-ol, Gallic acid, Trideca-1,11-diene-3,5,7,9-tetrayne, Lauric acid, Dimethoxyphenol, TLR4 was a core target of Ferullic acid, Gallic acid, Trideca-1,11-diene-3,5,7,9-tetrayne, TNF was a core target of Ferullic acid, Gallic acid, Trideca-1,11-diene-3,5,7,9-tetrayne, Dimethoxyphenol, CXCL8 was a core target of Trideca-1,11-diene-3,5,7,9-tetrayne and 7-Phenyl-hepta-2,4,6-triyn-2-ol and finally MMP9 was a core target of trideca-1,11-diene-3,5,7,9-tetrayne and dimethoxyphenol as its is evidenceced by their high binding energy values.

CHAPTER 01: INTRODUCTION

BACKGROUND OF THE STUDY

Malaria is an acute febrile illness. In non-immune individuals, symptoms are fever, headache, flu-like illness. It's caused basically by the plasmodium parasites which are of five species i.e. falciparum, vivax, malariae, ovale and knowlesi. However, the Malaria infections in Uganda are caused mainly by falciparum parasites up to 98%, followed by malariae and vivax, ovale being mild spread of malaria to the population. In Uganda, karamoja sub region is one of the highest malaria burden areas in Uganda with it's malaria parasite prevalence estimated to 34% amongst children under 5 years by statistics of 2021.Globally Uganda stands at position three among the highly infected countries i.e. Nigeria (26.6%), Democratic Republic of Congo (12.3%), Uganda (5.1%), Mozambique (4.1%), Burkina Faso (3.3%), Mali (3.1%), and Ghana (2.2%).About 96% of malaria deaths globally were in in 29 countries for just over half of all malaria deaths globally in 2021; Nigeria (31%), Democratic Republic of Congo (13%), Niger (4%), and Tanzania (4%)(Dang et al., 2016).

The national malaria treatment policy of Uganda strongly recommends the use of artemisinin-based combination therapy (ACT) as the first-line treatment for malaria, with a directive that emphasizes parasitological confirmation before administering therapy. Here are the anti-malarial drugs commonly used in Uganda: Artemether-Lumefantrine, the most commonly administered anti-malarial, with 88.3% prevalence in usage among children prior to hospital visits, Quinine is used at a lesser proportion, accounting for about 9.7% of usage. It is a traditional treatment for malaria and is also used as a second-line drug for the treatment of uncomplicated malaria and as the first-line treatment for severe malaria in Uganda. Artesunate although used less frequently (4.2%), artesunate is another anti-malarial mentioned in the studies, and it is also a part of the ACT regimen.Dihydroartemisinin PiperaquineDihydroartemisinin-piperaquine was used in 2.8% of cases according to the latest research.(Alebie et al., 2017)

However, Bidens pilosa, commonly known as blackjack, is a plant that is traditionally used for medicinal purposes including the treatment of malaria. Its anti-malarial properties are attributed to several biochemical compounds found within the plant. Active Chemical Components chemical of Bidens pilosa that have been associated with anti-malarial are Polyacetylenes which are bioactive compounds with significant anti-parasitic properties which can affect the parasite's life cycle, Flavonoids which are group of natural substances with variable phenolic structures found in plants. For example okanin and its derivatives, have shown antimalarial activity, Alkaloids that are naturally occurring organic compounds containing basic nitrogen atoms and finally essential Oils that have been associated with therapeutic effects, including potential anti-malarial properties. Mechanism of Action the exact mechanism whereby these components exert their anti-malarial effect is not fully understood, but it is believed to involve Inhibition of the growth and reproduction of the malaria parasite Plasmodium within the human body strengthening the immune system to combat the parasite more effectively. Clinical Evidence and usage while these components have shown promise in laboratory settings or in traditional use, it's important to note that the

effectiveness and safety of Bidens pilosa as an anti-malarial treatment have not been fully established in extensive clinical trials.(Dang et al., 2016)

Network pharmacology is an emerging method which not only combines network biology and poly-pharmacology to validate drug-actionable targets through computational software, but also explores potential mechanisms of drug therapeutic actions . Network pharmacology also provides a way of thinking about drug discovery while being able to understand the side effects and toxicity of drugs. This method has completely altered the approach to the definition, diagnosis, and treatment of diseases. The 3D modeling of the drug and protein receptor can be constructed by computer software, which can screen the optimal sites on the protein receptor for amino acid-ligand docking. In turn, these genetic proteins are inter-linked to disease, and thus a web of relationships between drugs and disease has been established. Furthermore, the strength of the association between pivotal genes and drugs can be confirmed by molecular docking. Therefore, in this study, we used network pharmacology and molecular docking to explore the specific molecular mechanisms of bidens pilosa for the treatment of malaria(Alebie et al., 2017)

1.1 PROBLEM OF THE STATEMENT

A large number of people especially children are dying slowly as a result of a common health/medical condition known as malaria which has affected their normal body functioning, reduction in their weight and deaths if not treated early.

The ministry of health together with the natural drug authority have recommended some medical health care for instance the use of artemisinin-based combination therapy and others. However, there is a prevalence of inappropriate use of anti-malarials which often involves self-medication and empiric prescription without parasitological confirmation which leads to issues such as drug resistance, which is a concern in many parts of Uganda.

Traditional medication (local herbs) has been the option to replace the drugs because of fear of their consequences. Bidens pilosa is one of the local plants used to treat malaria. Therefore the mechanism of components of bidens pilosa as an anti-malaria treatment needs to be identified.

1.2 PURPOSE OF THE STUDY

- To analyse the nature of phytochemical components of bidens pilosa
- To identify the potential anti-malarial mechanism of components of bidens pilosa

1.3 OBJECTIVES OF THE STUDY

- To determine the phytochemical components in bidens pilosa
- To discover the mechanism of components of bidens pilosa as an anti-malarial drug
- To demonstrate the mode of action of the phytochemical components found in bidens pilosa

1.4 SIGNIFICANCE

The research holds significant importance as it addresses the growing concern of malaria resistance to drugs available, bidens pilosa provides a natural and sustainable alternative to malaria, explores mode of action of the phytochemicals, enhances the effective treatment of malaria. By establishing the mode of action of the anti-malaria related phytochemicals in bidens pilosa, and economically reduces on the amount of money spent on purchase of anti-malaria related drugs

CHAPTER TWO: LITERATURE REVIEW

Malaria is an acute febrile illness. In non-immune individuals, symptoms are fever, headache, flu-like illness. It's caused basically by the plasmodium parasites which are of five species i.e. falciparum, vivax, malariae, ovale and knowlesi. However, the Malaria infections in Uganda are caused mainly by falciparum parasites up to 98%, followed by malariae and vivax, ovale being mild spread of malaria to the population. In Uganda, karamoja sub region is one of the highest malaria burden areas in Uganda with it's malaria parasite prevalence estimated to 34% amongst children under 5 years by statistics of 2021.Globally Uganda stands at position three among the highly infected countries i.e. Nigeria (26.6%), Democratic Republic of Congo (12.3%), Uganda (5.1%), Mozambique (4.1%), Burkina Faso (3.3%), Mali (3.1%), and Ghana (2.2%).About 96% of malaria deaths globally were in in 29 countries for just over half of all malaria deaths globally in 2021; Nigeria (31%), Democratic Republic of Congo (13%), Niger (4%), and Tanzania (4%).

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Network pharmacology is an emerging method which not only combines network biology and poly-pharmacology to validate drug-actionable targets through computational software, but also explores potential mechanisms of drug therapeutic actions . Network pharmacology also provides a way of thinking about drug discovery while being able to understand the side effects and toxicity of drugs. This method has completely altered the approach to the definition, diagnosis, and

treatment of diseases. The 3D modeling of the drug and protein receptor can be constructed by computer software, which can screen the optimal sites on the protein receptor for amino acid-ligand docking. In turn, these genetic proteins are inter-linked to disease, and thus a web of relationships between drugs and disease has been established. Furthermore, the strength of the association between pivotal genes and drugs can be confirmed by molecular docking. Therefore, in this study, we used network pharmacology and molecular docking to explore the specific molecular mechanisms of bidens pilosa for the treatment of malaria.

Among the Asteraceae species, Bidens pilosa is one of the most promising and potent anti-malaria botanicals, as it shows strong inhibition against parasitemia in vitro cultures. In earlier reports demonstrated that the plant has low in vitro activities against Plasmodium berghei. More importantly, dried whole plant materials of Bidens pilosa extracted with ethanol, butanol, and chloroform, show a 90 % inhibition against the in vitro growth of the deadly malarial strain Plasmodium falciparum at 50 lg/ml. (Tang et al., 2022).The ethanolic extract of the root exhibits a much higher inhibition in mice infected with Plasmodium berghei than the whole plant, leaf and stem extracts. The chloroform fractions of the root exert an 86 % suppression of Plasmodium falciparum growth in vitro. Another trial in mice confirmed this effect in vivo, with a reduction in parasitemia of up to 60 % in mice infected with Plasmodium berghei at 250–500 mg/kg. Chloroquine- or mefloquineresistant Plasmodium falciparum strains are susceptible to Bidens pilosa (IC50 = 10.4–49.8 lg/mL) in vitro. Interestingly, extracts from plants cultivated under standardized conditions are less active in comparison with wild plants (Andrade-Neto et a falciparum (NF54 strain) in human blood is significantly inhibited at IC50 = 32.8 lg/mL using the hexane extract of Bidens pilosa leaves in vivo assays . The chloroform ether and ether methanol (1:1) fractions obtained from the root extract provide both a polyacetylenic ingredient, compound Phenylheptatriyne and 2 methoxylated flavonoids as the major compounds Centaureidin and Digitoflavone. These show strong anti-malarial activities in vivo and are bioactive towards Plasmodium. (Swain & Padhy, 2016)The strong anti-malarial ability of Bidens pilosa is likely due to its abundant production of polyacetylenes and flavonoids. For instance, compound Phenylheptatriyne is one of the major polyacetylenic compounds occurring at high concentrations, which is bioactive towards several malarial strains and shows potent inhibitory activity against Plasmodium falciparum, with IC50 = 6.1 lg/mL. However, Bidens pilosa likely has low activities because the active compounds are rapidly degraded during fractionation or at storage. The biological activities of the polyacetylenes are dependent on light for their toxicity and ultraviolet light for the expression of their activities. Another polyacetylenic constituent is compound (R)-1,2-Dihydroxytrideca-3,5,7,9,11-pentayne contained in the aerial parts of the plant, which also exhibits complete in vitro inhibition of Plasmodium falciparum at 1 lg/mL and causes significant suppression of the Plasmodium berghei strain at 0.8 mg/kg in infected mice over 4 days This compound is stable in the organic solvents methanol or ethyl acetate, but unstable in the solid state Compound Campestrol is present in all parts of Bidens pilosa and is very active in mice infected by Plasmodium berghei at a dose of 15 mg/kg, inhibiting parasitemia by up to 58 % at 8 days after parasite inoculation. However, this high dose raises the question of its practical relevance. Interestingly, compound Okanin 4 -glucoside, contained in the aerial parts of the plant is inhibitory also for leishmania. This suggests that this compound should be further investigated for the development of new anti-malarial and anti-leishmania drugs in the future. *Bidens pilosa* has potential beneficial therapeutic actions that can be used in the management of malaria and possibly even of leishmania(Hao & Xiao, 2014)

CHAPTER THREE: METHODOLOGY AND MATERIALS

- **PUBCHEM**: Online software that enables chemists identify physiochemical properties in *bidens pilosa* in addition to google scholars.

- **CANONICAL SMILES**: Simplified molecular-input line-entry system which converts 3-D structures into a line of symbols which computer software can understand foreample for benzoic acid is OC(=O)C1=CC=CC=C1

- **MOLECULAR OPERATING ENVIRONMENT**: Software used to predict the structure of molecules using the smiles and docking of the protein surfaces to the gene targets.(Shoichet, 2007)

- **SWISSADME**: compute physicochemical descriptors as well as pharmacokinetic properties, drug like nature and medicinal chemistry friendliness of small molecules to support drug discovery.

- **SWISSTARGET PREDICTION DATABASE**: Used estimate the most probable macromolecular targets of a small molecule, assumed as bioactive**.**

- **OMIM, DISGNET AND GENECARDS DATABASES**

- **INTERACT VENN DATABASE** is an interactive visualization tool for analyzing lists of elements using Venn diagrams. The web tool supports up to six different sets.

- **STRINGS DATABASE** Protein-Protein Interaction Networks Functional Enrichment Analysis

- **CYTOSCAPE APP** is an open source software platform for visualizing complex networks and integrating these with any type of attribute data**.**

CHAPTER FOUR: METHODOLOGY AND MATERIALS

2.1 Network pharmacology

SWISSADMETox-analysis of active compounds

Oral bioavailability radars were built for the most active compounds (IC50≤1μM), using SwissADME. This analysis describes the drug-likeness of a molecule using 6 properties of lipophilicity, size, polarity, insolubility, saturation, and fexibility . For each of these parameters, the radar represents the physicochemical space within the best range of lipophilicity (XLOGP3 –0.7 and+5.0), molecular weight (150–500 g/mol), polarity (Topological PSA, 20–130 Å, solubility (log S−6 and 0), saturation (≥0.25), and fexibility (≤9 rotatable bond) .No compounds showed deviations on the lipophilicity values as well on the saturation;. Compounds should comply with all 6 properties to be considered orally active. It was observed that physicochemical parameters of the most active compounds seemed to have lower values of lipophilicity and solubility than those for inactive compounds except for polarity (PSA) and molecular refractivity (MR) values; however, this trend was not signifcant. The computed ADME, physicochemical properties, drug-likeness, and medicinal chemistry predictions are summarized in Table 3. According to the results, all compounds showed high GI absorption except compounds T, U and V, as well most compounds showed high permeation in the BBB except A, B, C, D, R and T . None of the compounds interacted with P-glycoprotein (Pgp) a transmembrane drug transporter. Most compounds interact with the cytochrome isoforms, indicating potential drug interactions and biotransformation mediated by the interacting cytochrome, Compounds B, C, D, E, J, L, and O showed no violations in all 5 rule flters: Lipinski, Ghose, Veber, Egan, and Muegge. All the compounds showed no violations for Veber and Egan rules, whereas 24 compounds had violations of Muegge, and 14 showed violations of Ghose. For the medicinal chemistry parameters, the bioavailability score for all compounds suggests>10% bioavailability in rats or cells permeability Compounds were of easy synthesis as judged by the synthetic accessibility index ranging from 3.26 to 4.46 as illustrated in **table 1** .(Li et al., 2022)

A. (2R)-trideca-3,5,7,9,11-pentayne-1,2-diol
B. 2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one
C. 5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-3,6-dimethoxychromen-4-one
D. (E)-3-(3,4-dihydroxyphenyl)-1-(2,3,4-trihydroxyphenyl)prop-2-en-1-one
E. 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane
F. 1-thiophen-2-ylethanone
G. Ferullic acid
H. 2-hydroxy-6-methylbenzaldehyde
I. 1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol
J. 2,3-dimethoxyphenol
K. ethyl 3-(3,4-dihydroxyphenyl)prop-2-enoate
L. 3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid
M. 3,4,5-trihydroxybenzoic acid
N. 1-methyl-4-prop-1-en-2-ylcyclohexa-1,3-diene
O. 3,4-dihydroxybenzoic acid
P. salicylic acid

Q. 2-methyl-5-propan-2-ylcyclohexa-1,3-diene
R. 1,6-dimethyl-4-propan-2-yl-3,4,4a,7,8,8a-hexahydro-2H-naphthalen-1-ol
S. 3,7-dimethylocta-1,6-dien-3-ol
T. 4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-ol
U. Lauric acid
V. 2-(4-methylphenyl)propan-2-ol
W. 4-ethenyl-2-methoxyphenol

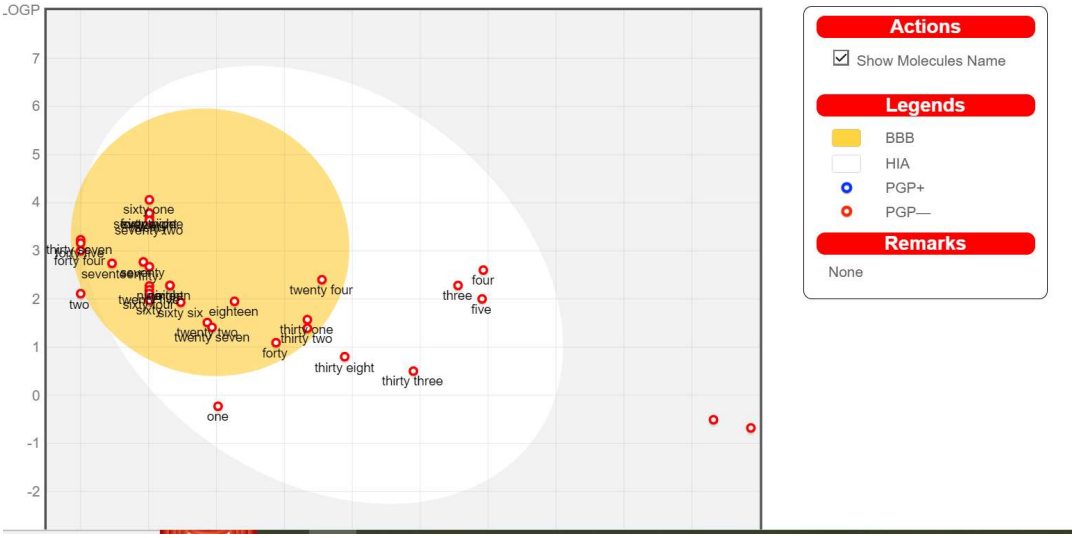
Table 1 Physicochemical properties, ADME, and medicinal chemistry predictions of active compounds (IC50≤1 µM)

CPD	MW	MR	ESOL LogS	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP3A4 inhibitor	Lipinski	Ghose	Veber	Egan	mueggge	Bioavailability Score
A	196.2	57.74	-1.68	High	No	No	No	No	No	No	0	0	0	0	1	0.55
B	286.24	76.01	-3.71	High	No	No	Yes	No	No	Yes	0	0	0	0	0	0.55
C	360.31	93.47	-4.02	High	No	No	Yes	No	Yes	Yes	0	0	0	0	0	0.55
D	288.25	76.36	-3.41	High	No	No	Yes	No	Yes	Yes	0	0	0	0	0	0.55
E	154.25	47.12	-2.52	High	Yes	No	No	No	No	No	0	1	0	0	2	0.55
F	126.18	34.	-1.81	High	Yes	No	No	No	No	No	0	3	0	0	1	0.55

		51														
G	220.35	70.2	-2.68	High	Yes	No	No	No	No	No	0	0	0	0	1	0.55
H	136.15	38.82	-2.28	High	Yes	No	Yes	No	No	No	0	3	0	0	1	0.55
I	154.25	46.6	-2.51	High	Yes	No	No	No	No	No	0	1	0	0	2	0.55
J	154.16	41.45	-1.82	High	Yes	No	No	No	No	No	0	1	0	0	1	0.55
K	208.21	56.28	-2.78	High	Yes	No	No	No	No	No	0	0	0	0	0	0.55
L	194.18	51.63	-2.11	High	Yes	No	No	No	No	No	0	0	0	0	1	0.85
M	170.12	39.47	-1.64	High	No	No	No	No	No	Yes	0	2	0	0	1	0.56
N	134.22	46.65	-2.52	Low	Yes	No	No	No	No	No	0	1	0	0	2	0.55
O	154.12	37.45	-1.86	High	No	No	No	No	No	Yes	0	3	0	0	1	0.56

P	**138.12**	**35.42**	**-2.5**	**High**	**Yes**	**No**	**No**	**No**	**No**	**No**	**0**	**3**	**0**	**0**	**1**	**0.85**
Q	**136.23**	**45.222**	**-2.53**	**Low**	**Yes**	**No**	**No**	**No**	**No**	**No**	**1**	**1**	**0**	**0**	**2**	**0.55**
R	**136.23**	**47.12**	**-2.64**	**Low**	**Yes**	**No**	**No**	**No**	**No**	**No**	**0**	**1**	**0**	**0**	**2**	**0.55**
S	**222.37**	**70.72**	**-3.26**	**High**	**Yes**	**No**	**No**	**Yes**	**No**	**No**	**0**	**0**	**0**	**0**	**1**	**0.55**
T	**154.25**	**50.444**	**-2.4**	**High**	**Yes**	**No**	**No**	**No**	**No**	**No**	**0**	**1**	**0**	**0**	**2**	**0.55**
U	**152.23**	**46.38**	**-2.77**	**High**	**Yes**	**No**	**No**	**No**	**No**	**No**	**0**	**1**	**0**	**0**	**2**	**0.55**
V	**222.37**	**70.466**	**-3.29**	**High**	**Yes**	**No**	**No**	**No**	**No**	**No**	**0**	**0**	**0**	**0**	**1**	**0.55**
W	**150.22**	**47.03**	**-2.36**	**High**	**Yes**	**No**	**Yes**	**No**	**No**	**No**	**0**	**1**	**0**	**0**	**2**	**0.55**
X	**150.17**	**45.055**	**-2.81**	**High**	**Yes**	**No**	**Yes**	**No**	**No**	**No**	**0**	**1**	**0**	**0**	**1**	**0.55**

BOILED EGG SHOWING RESULTS FROM SWISSADME DATABASE



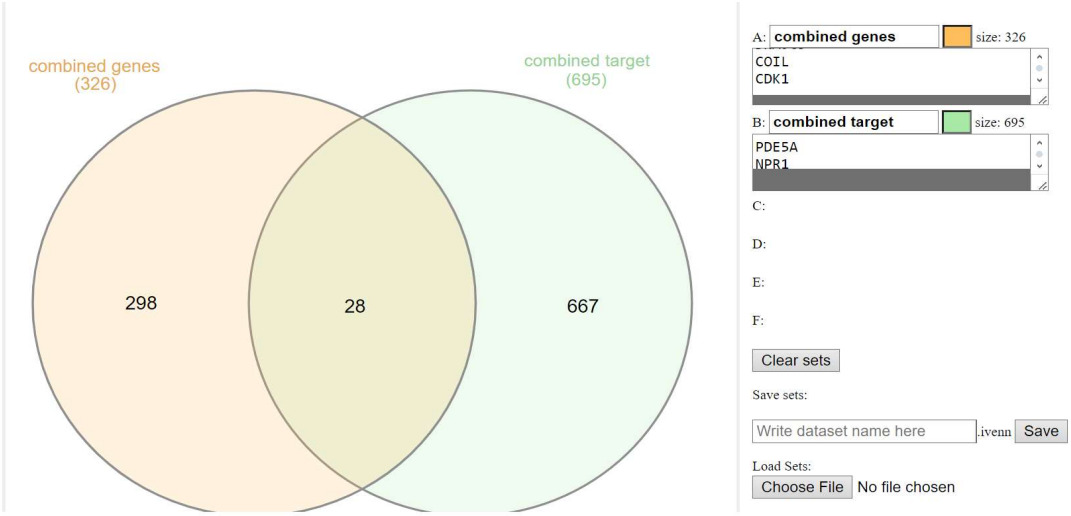
Target prediction for anti-malaria components in *bidens pilosa* using swisstargetprediction

The workflow of this analysis starts from Isomeric SMILES of bidens pilosa which were obtained from PubChem, the world's largest chemical information base where we could get chemical structures, chemical properties, biological activities of small molecules. The swisstargetprediction databases were screened to identify potential target genes. This databases were designed to predict drug-related genes "Human Species" was set as a requirement for this analysis. Following the removal of duplicates, the selected targets were standardized using the UniProt database. The genes screened from this databases under certain conditions were the genes associated with the therapeutic effects of bidens pilosa. Phytochemicals of bidens pilosa may exert its therapeutic effects towards diseases through interactions between these genes.(Dongmo et al., 2023)

Identification of potential targets in malaria and intersection genes

Malaria-related genes were recognized using three genetic databases, namely, DisGenet, GeneCards, Online Mendelian Inheritance in Man (OMIM). Based on the rankings, we searched for mutated genes that are more likely to be involved in the development and progression of malaria, and used UniProt to normalize these disease-associated genes. The drug and disease genes were subsequently mapped, and the mapping results were imported into the interact venn to obtain intersection genes. Therapeutic effects of bidens pilosa on malaria were likely to be mediated by modulation of these intersecting genes and the interaction is illustrated in the figure 4

FIGURE 4 SHOWING RESULTS OF THE INTERACT VENN DATABASE



And the genes obtained in the intersection are **G6PD, TNF, NOS2, TLR9, TLR4, HMOX1, ALB, MIF, CXCL8, IL2, GSR, ELANE, ACP1, ADRB2, FLT1, ADA, ABCB1, MMP9, PTGS2, ADORA2A, F3, NOS1, CASP3, HPGDS, HPGD, GSTK1, VDR** and **CDK1**(Asiamah et al., 2023)

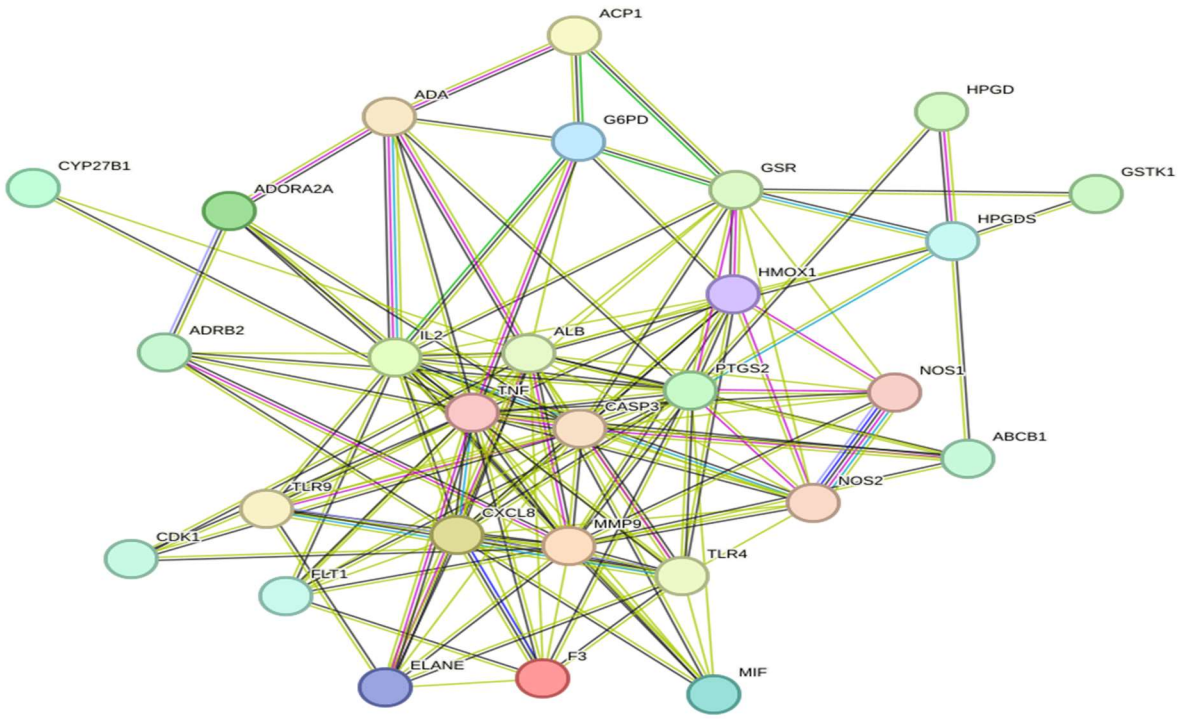
2.1.4 Drug-target-pathway network construction

The drug-target-pathway network was established by introducing intersection genes and KEGG signaling pathway items into Cytoscape 3.9.1. Cytoscape is a platform that integrates complex network structures with data and presents them in graphical form .The cellular nodes represent bidens pilosa, intersection genes, or pathways, while the connecting lines between different nodes represent genes involved in different pathways.(Alzain et al., 2022)

Protein-protein interaction (PPI) network construction and visualization using strings database

PPI is a method utilized to study the mechanism through which proteins function harmoniously in cells. Using the STRING database, we constructed the PPI network with the species limited to "Homo sapiens" and a medium confidence score of 0.4 to ensure more protein-protein information would be included. The PPI network was updated after removing disconnected nodes. The tab-separated values (tsv) format file was downloaded and imported into Cytoscape 3.9.1 for subsequent visualization. The strength of interactions between proteins in Cytoscape would be calculated in order to screen for core targets as in figure 1 below.(Nwonuma et al., 2023)

The figure 1 below shows the interaction of prorein-protein interaction of genes and targets using strings database



Screening of core targets using cytoscape

Using the "Cytohubba" plug-in in Cytoscape 3.9.1, we determined the core targets according to their degree values. "CytoNCA" was a plug-in for calculating the strength of interactions between proteins, some proteins with higher interaction strengths with other proteins could be screened out as core targets. Several methods for calculating the strength of protein associations were included in this plug-in and the methods chosen among the many are degree, MNC, and MCC have been chosen as methods for screening core targets where the top 10 gegnes were selected according to each method. The intersecting genes for each method were copied to the interact venn for three sets as **Degree**, **MNC**, and **MCC** to obtain the intersection of genes which were selected as core targets as in **table 2**. (Li et al., 2022)

TABLE 2 SHOWING RESULTS OF MNC, MCC AND DEREE

MNC	**MCC**	**DEGREE**
TNF	ALB	TNF
ALB	TNF	ALB
PTGS2	PTGS2	PTGS2
MMP9	MMP9	MMP9

CASP3	CXCL8	CASP3
IL2	IL2	IL2
CXCL8	CASP3	HMOX1
HMOX1	TLR4	CXCL8
TLR4	HMOX1	TLR4
GSR	NOS2	GSR

The intersection of the three sets were obtained as **ALB, TNF, PTGS2, IL2, MMP9, CASP3, TLR4, CXCL8, HMOX1** from the interact venn as illustrated in the figure 5 below

FIGURE 5 SHOWING THE RESULTS OF INTERACT VENN FOR MCC, MNC AND DEGREE

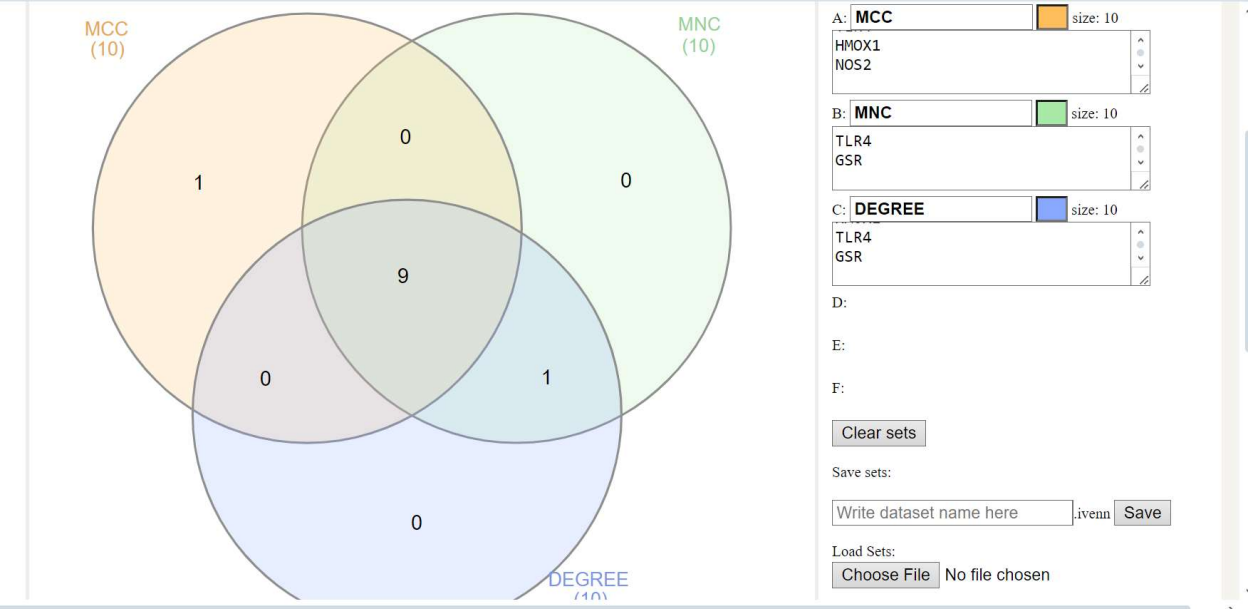
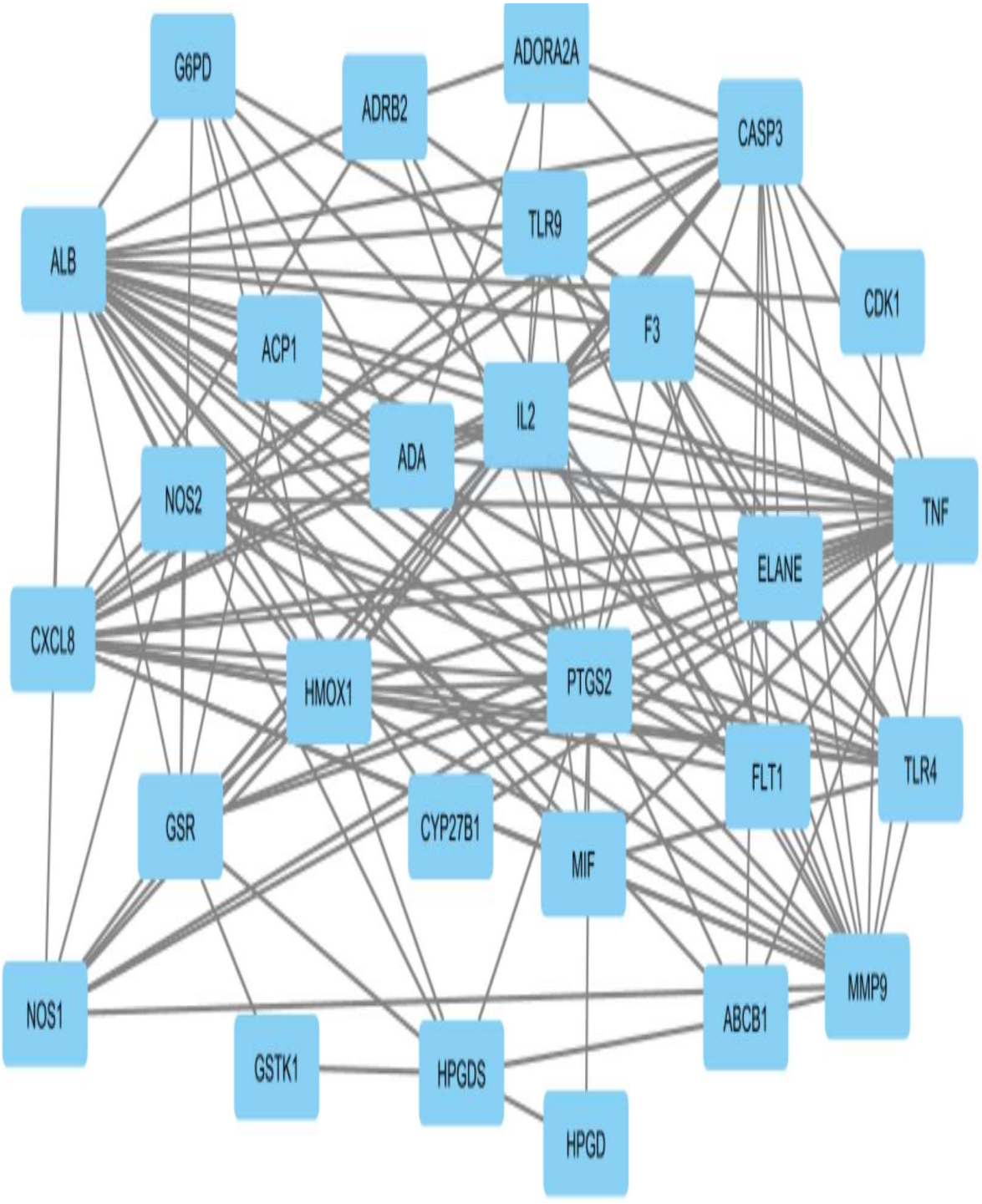


Figure 2 below shows visualization of data imported from strings database to cytoscape



Molecular docking between malaria genes and targets from bidens pilosa

We sought to further understand the relationship between candidate proteins and malaria, as well as their mechanism of action. Therefore, molecular docking was performed to determine the strength of the interaction between receptors and ligands. The SDF (Structural Data File) of malaria was downloaded from PubChem, and the Protein Data Bank (PDB) database was used to obtain the SDF format file of the original ligand. Moreover, the pdb format files of receptor proteins were obtained from the PDB database **(Table 3).** The SDF format files of bidens pilosa and the original ligand were transformed into mol2 format The receptor proteins were introduced into molecular operating environment for dehydrating and deligand. Thereafter, we modified the receptor proteins with hydrogenation in molecular operating environment. Setting torsion for ligand and outputting ligand as mbd format. The pdb format files of receptor proteins and ligands were re-imported into molecular operating environment

Add hydrogens and partial charges to the system with the Protonate3D application which performs the calculation and gives the progressive reports written to the MOE window. Thereafter the **Dock** panel was opened and setup the docking options for this particular run with all the Docking parameters as follows: Verify that the **Receptor** pull-down menu is set to **Receptor+Solvent** and that the **Site** pull-down menu is set to **Ligand Atoms** (the residues close to the ligand atoms will define the docking site), Use the default **Placement** method: **Triangle Matcher**, Set the first scoring function, **Rescoring 1** to the default **London dG** and set the **Retain** dropdown to 10, Set the **Refinement** to **Forcefield.** The docked poses will be energy minimized in the receptor pocket,Set the refinement scoring function, **Rescoring 2** to **None** and set the **Retain** dropdown to 10. With this setting, the final refined poses are ranked by the MM/GBVI binding free energy estimation. Finally docking was performed, the docked poses and scores were written and recorded to the 'dock.mdb' output database.(zhao et al., 2020)

Protein Active Site was set by making use of the visualization tools in LigX to help decide what modifications to make to ligand, for example **Surfaces and Maps** panel which calculates displays a number of surfaces and maps to analyze the active site and furthermore calculates an **Electrostatic Map** from the receptor atoms and displays the preferred locations for hydrophobic entities as well as hydrogen bond donors and acceptors of the ligand, the Electrostatic Map displays a number of locations where adding hydrophobic groups or hydrogen bond donors or acceptors would be energetically favorable. To clear up the view, hide the donor and acceptor regions in the **Surfaces and Maps** .(Asiamah et al., 2023)

TABLE 3.

Details of the protein targets in the PDB database

TARGETS	PDB ID	METHOD	RESLUTION	R-VALUE FREE	R-VALUE WORK	R-VALUE OBSERVED

TNF	4E4S	X-RAY DIFFRACTION	1.95 Å	0.178	0.147	0.148
CASP3	2MUE	SOLUTION NMR	**…….**	**……**	**…….**	**……**
TLR4	5UC8	X-RAY DIFFRACTION	2.00 Å	0.251	0.196	0.198
HMOX1	7KIY	ELECTRON MICROSCOPY	2.92 Å	**Aggregation State:** PARTICLE	**Reconstruction Method:** SINGLE PARTICLE	**…….**
CXCL8	8IC0	ELECTRON MICROSCOPY	3.41 Å	**Aggregation State:** PARTICLE	**Reconstruction Method:** SINGLE PARTICLE	**…….**
PTGS2	7MRW	ELECTRON MICROSCOPY	3.72 Å	**Aggregation State:** PARTICLE	**Reconstruction Method:** SINGLE PARTICLE	**……..**

MOLECULAR DOCKING RESULTS USING MOLECULAR OPERATING ENVIRONMENT

From the results in cytoscape the following genes to be used under molecular docking softwares to determine the mechanism of action:

TNF(Tumor necrosis factor): it's the gene associated with **severe malaria**

PTGS2(Prostaglandin- Endoperoxide Synthase 2): Is a gene that encodes a protein called cyclooxygenase-2, which is involved in inflammatory response to malaria. As well **CXCL8** but uses interleukin 8. **Once the malaria parasites are detected, the chemokines and cytokines are synthesized**

MMP9: Is a gene that encodes a protein **matrix metallopeptidase 9**, which is involved in the **breakdown of the blood-brain barrier during cerebral malaria**(Wang et al., 2022)

TLR4: It encodes a protein called **toll-like receptor 4**, which is involved in the **recognition of malaria parasites** and the **activation of the innate immune response**(Nasim Habibi, parham Rouhi, 2017)

However **ALB(Albumin)**, **IL2(Interleukin 2)** and **HMOX1(heme oxygenase-1)** have less impact on malaria parasites

47- Ferullic acid

24- 7-Phenyl-hepta-2,4,6-triyn-2-ol

8-Gallic acid

22- Trideca-1,11-diene-3,5,7,9-tetrayne

46- Lauric acid

20- Dimethoxyphenol

RESULTS FROM CXCL8 TARGET

RESULTS OF COMPOUND Trideca-1,11-diene-3,5,7,9-tetrayne

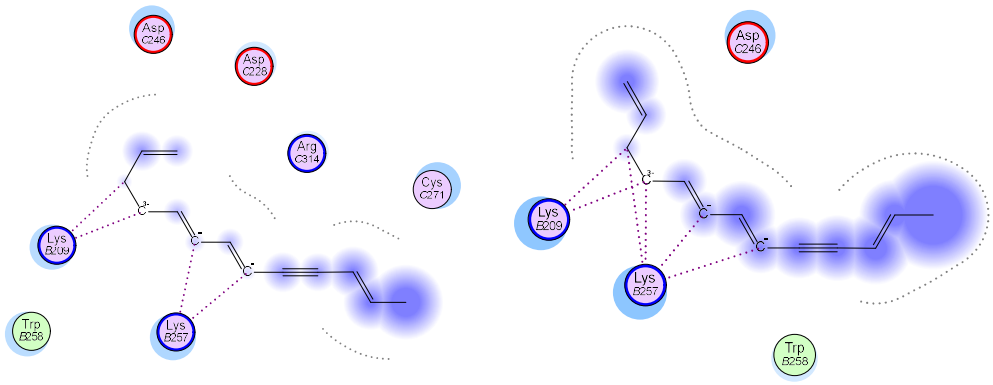
COMPOUND 22	**S**	**E score 1**	**E refine**
182	-19.6057	-13.5481	-115.6034
183	-19.4106	-13.5840	-51.1507

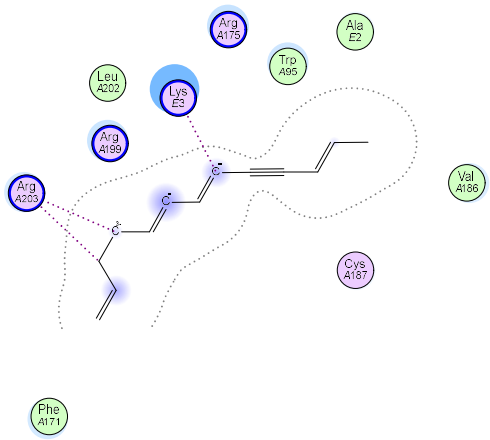
184	-18.5745	-14.2098	-55.6522
185	-14.6868	-11.2782	-89.9491
186	-14.5404	-12.6917	-108.5120
187	-14.5367	-12.4595	-53.5949
188	-13.5839	-12.0126	-74.4488
189	-13.5749	-11.1099	-55.1530
190	-12.8646	-11.1290	-45.8812
191	-12.7316	-12.0173	-65.0719

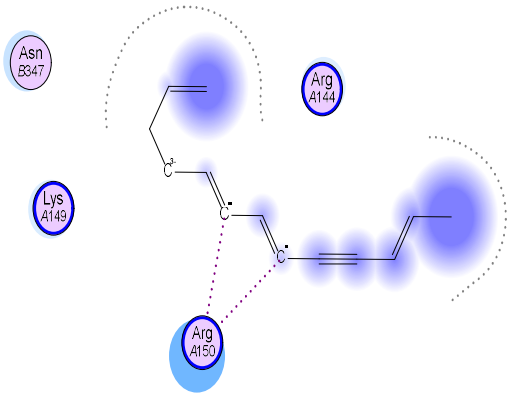
RESULTS FROM COMPOUND 7-Phenyl-hepta-2,4,6-triyn-2-ol

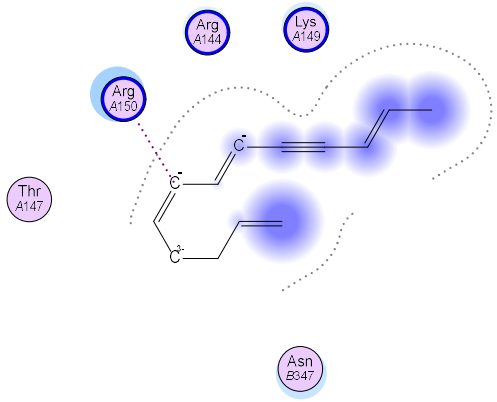
COMPOUND	S	E SCORE	E REFINE
351	**-10.4270**	**-9.8385**	**-42.2056**

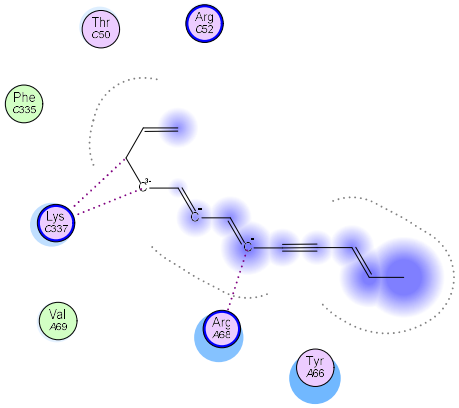
LINGAND INTERACTIONS FOR CXCL8 TARGET WITH COMPOUND Trideca-1,11-diene-3,5,7,9-tetrayne

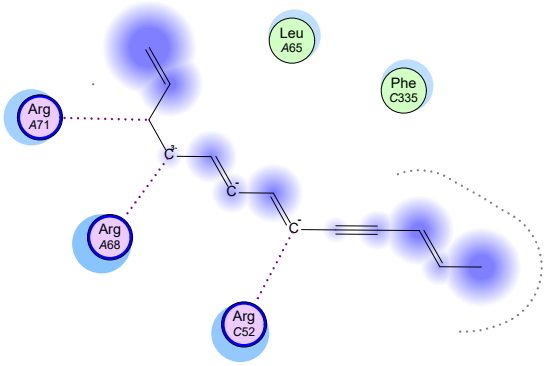


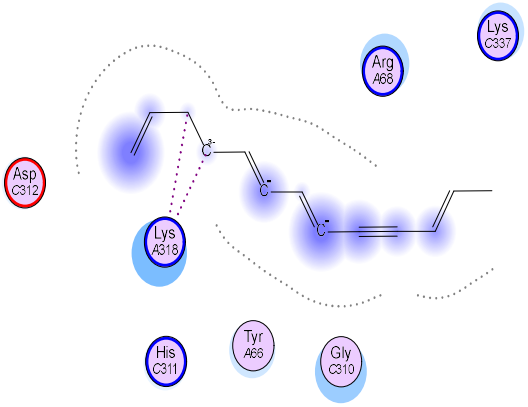

Arg A175
Ala E2
Trp A95
Leu A202
Lys E3
Arg A199
Arg A203
Val A186
Cys A187
Phe A171

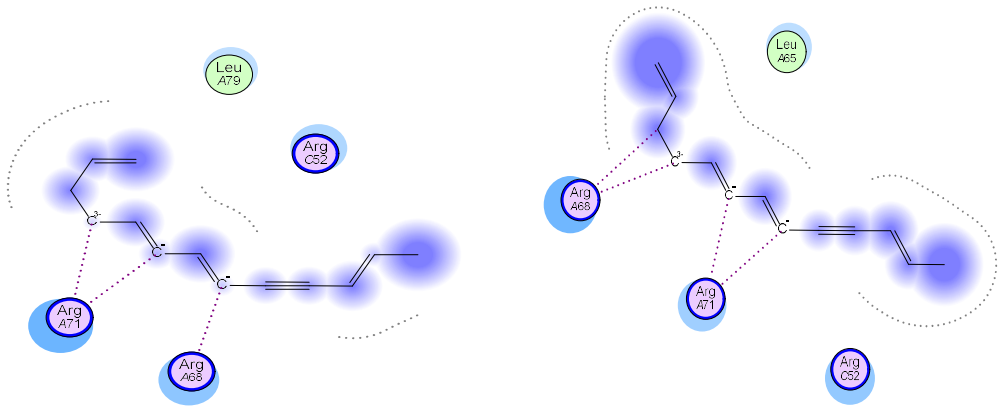

Asn B347
Arg A144
Lys A149
Arg A150


Arg A144
Lys A149
Arg A150
Thr A147
Asn B347

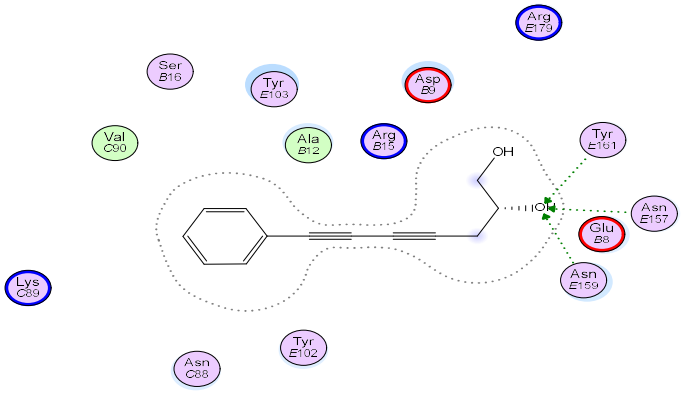

Thr C50
Arg C52
Phe C335
Lys C337
Val A69
Arg A68
Tyr A66


Leu A65
Phe C335
Arg A71
Arg A68
Arg C52


Lys C337
Arg A68
Asp C312
Lys A316
Tyr A66
His C311
Gly C310



Ligand interactions of CXCL8 with compound 1-Phenyl-1,3-diyn-5-en-7-ol-acetate



RESULTS FROM TLR4 TARGET

COMPOUND Gallic acid

COMPOUND **Gallic acid**	**S**	**E score 1**	**Energy**
61	-9.4899	-9.4899	-99.1394
62	-9.3867	-9.3867	-99.1439

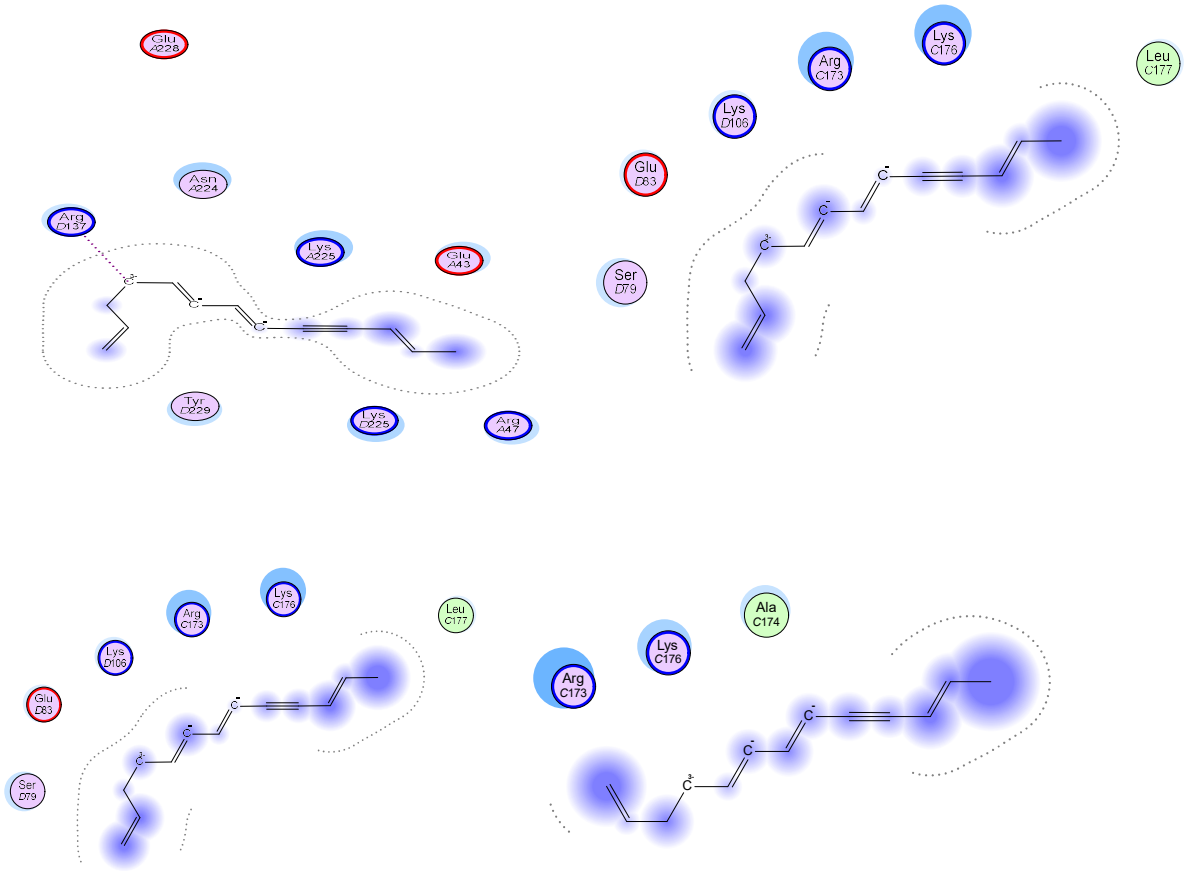
COMPOUND 47

441	**-11.4568**	**-11.4568**	**-99.1690**
441	**-10.3077**	**-10.3077**	**-99.1667**

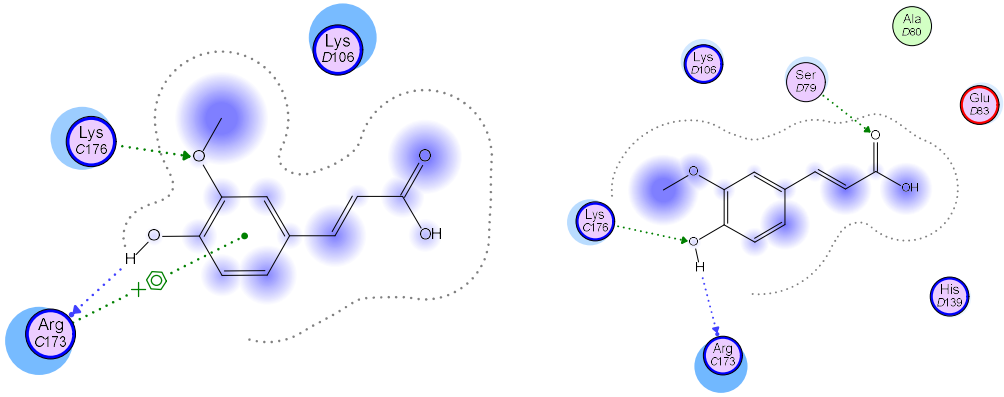
COMPOUND Trideca-1,11-diene-3,5,7,9-tetrayne

191	**-15.0985**	**-15.0985**	**-65.0696**
192	**-13.8105**	**-13.8105**	**-64.9986**
193	**-13.5938**	**-13.5938**	**-65.0581**
194	**-13.2614**	**-13.2641**	**-65.0798**
195	**-13.1286**	**-12.9820**	**-65.1168**
196	**-12.9820**	**-12.9820**	**-65.1136**
197	**-12.9655**	**-12.9655**	**-65.1095**
198	**-12.8958**	**-12.8958**	**-65.1139**
199	**-12.8796**	**-12.8796**	**-65.0778**
200	**-12.8273**	**-12.8273**	**-65.0999**

Lingand interactions for compound Trideca-1,11-diene-3,5,7,9-tetrayne with TLR4



Lingand interactions of compound 47 with TLR4



RESULTS FROM TNF TARGET WITH COMPONENTS IN BIDENS PILOSA

COMPOUND Gallic acid

COMPOUND	S	E SCORE	ENERGY
61	-14.2941	-14.2941	-94.8302
62	-12.6919	-12.6919	-94.8301
63	-12.5430	-12.5430	-94.8301
64	-12.9847	-12.2847	-94.8302
65	-12.2546	-12.2546	-94.8302
66	-12.1023	-12.1023	-94.8302
67	-11.9901	-11.9901	-94.8301
68	-11.9770	-11.9770	-94.8302
69	-11.8115	-11.8115	-94.8301
70	-11.7078	-11.7078	-94.8301

COMPOUND Trideca-1,11-diene-3,5,7,9-tetrayne

191	-15.3682	-15.3682	-65.0383
192	-14.8268	-14.8268	-65.0630
193	-14.5939	-14.5939	-65.0524
194	-14.2604	-14.2604	-65.0662

195	**-13.7378**	**-13.7378**	**-65.0452**
196	**-12.8384**	**-12.8384**	**-65.9445**
197	**-12.7467**	**-12.7467**	**-65.0085**
198	**-12.127**	**-12.6127**	**-65.0342**
199	**-12.2990**	**-12.2990**	**-65.0501**
200	**-12.1353**	**-12.1353**	**-65.0519**

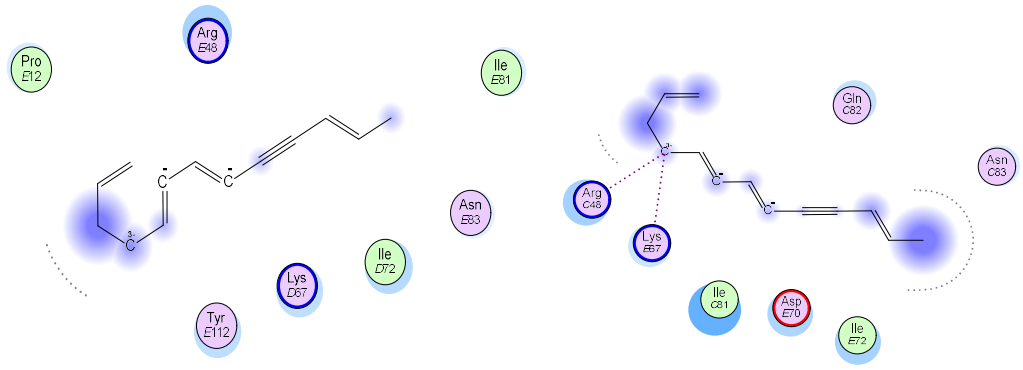
COMPOUND 47

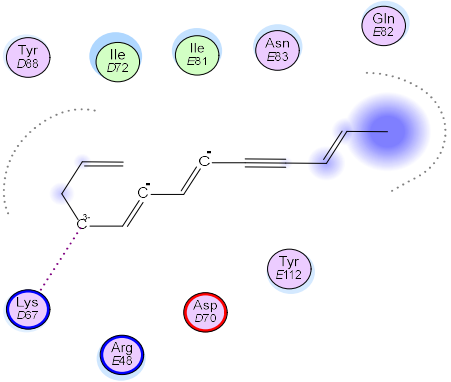
441	**-12.1443**	**-12.1443**	**-99.1391**
442	**-11.3499**	**-11.3499**	**-99.1645**
443	**-11.0828**	**-11.0828**	**-99.1638**
444	**-10.8603**	**-10.8603**	**-99.1664**
445	**-10.7225**	**-10.7225**	**-99.1626**
446	**-10.4361**	**-10.4361**	**-99.1708**
447	**-10.3666**	**-10.3666**	**-99.1395**
448	**-10.2117**	**-10.2177**	**-99.1437**
449	**-10.1886**	**-10.1886**	**-99.1419**
450	**-10.1569**	**-10.1569**	**-99.1659**

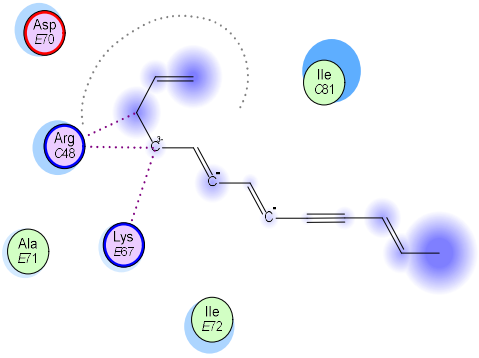
COMPOUND 20

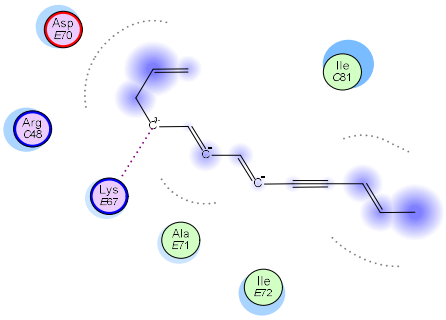
171	**-10.5266**	**-10.5266**	**-77.9246**
172	**-10.4820**	**-10.4820**	**-77.9792**
173	**-10.3612**	**-10.3612**	**-77.9743**
174	**-10.2065**	**-10.2065**	**-77.9787**
175	**-10.0525**	**-10.0525**	**-77.9802**

Lingand interactions for compound Trideca-1,11-diene-3,5,7,9-tetrayne with TNF

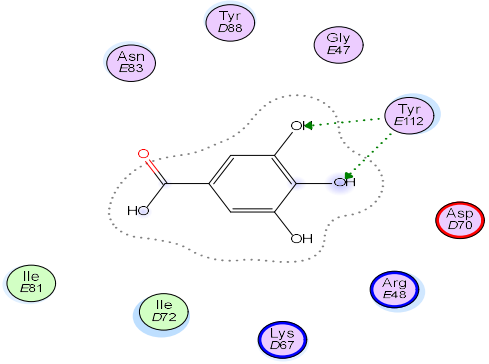


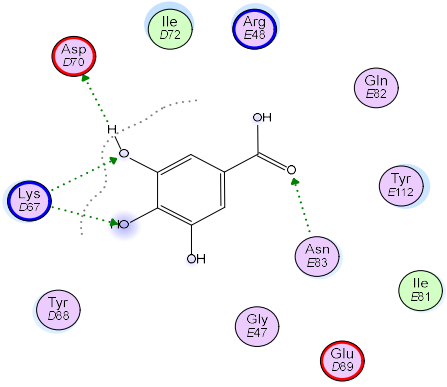


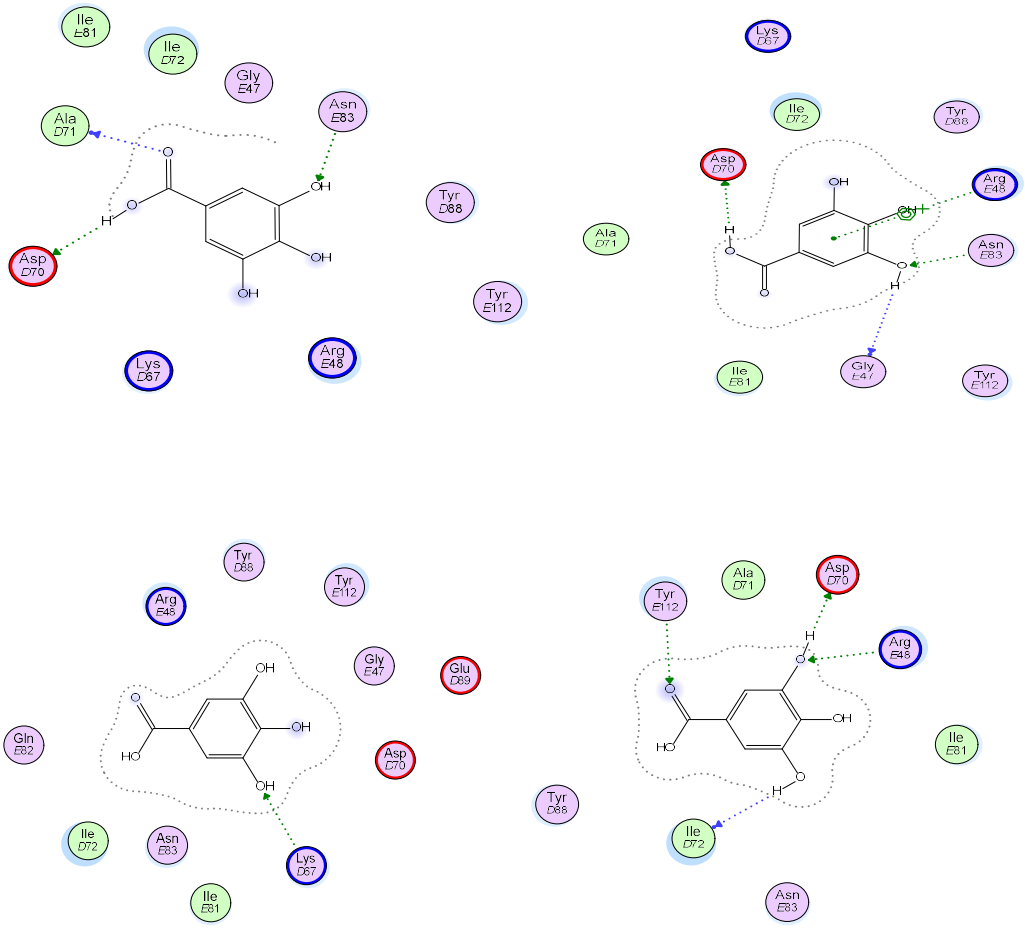




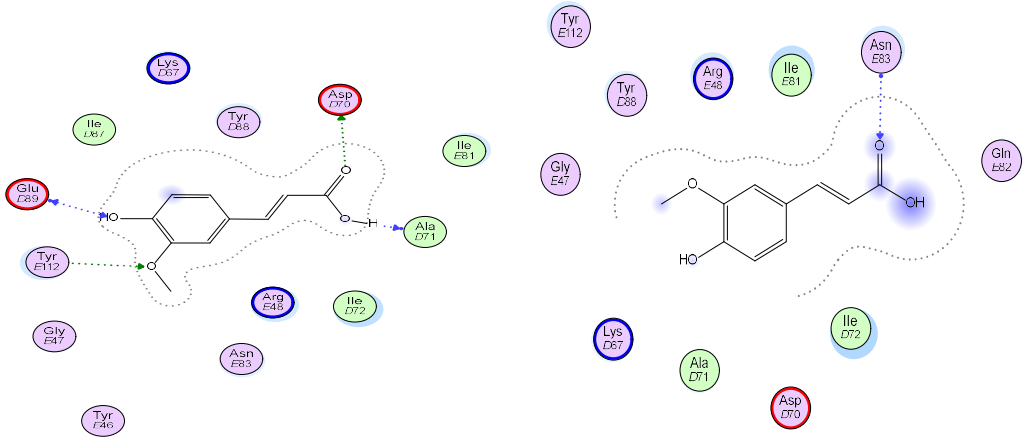
COMPOUND Gallic acid






Ile E81
Ile D72
Gly E47
Asn E83
Ala D71
Tyr D88
Asp D70
Tyr E112
Lys D67
Arg E48
Lys D67
Ile D72
Tyr D88
Asp D70
Arg E48
Ala D71
Asn E83
Ile E81
Gly E47
Tyr E112
Tyr D88
Tyr E112
Arg E48
Gly E47
Glu D89
Gln E82
Asp D70
Ile D72
Asn E83
Lys D67
Ile E81
Ala D71
Asp D70
Tyr E112
Arg E48
Ile E81
Tyr D88
Ile D72
Asn E83

COMPOUND 47


Lys D67
Asp D70
Ile E81
Tyr D88
Ile D72
Glu D89
Ala D71
Tyr E112
Arg E48
Ile D72
Gly E47
Asn E83
Tyr F46
Tyr E112
Arg E48
Ile E81
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Tyr D88
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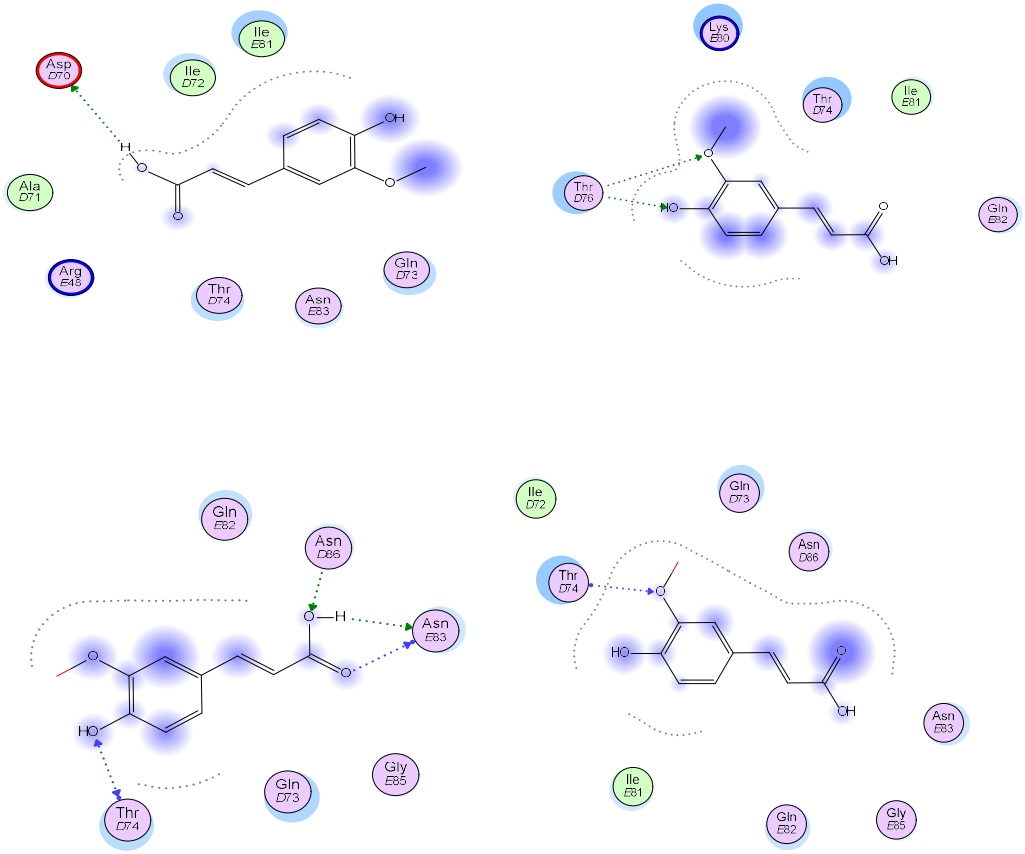
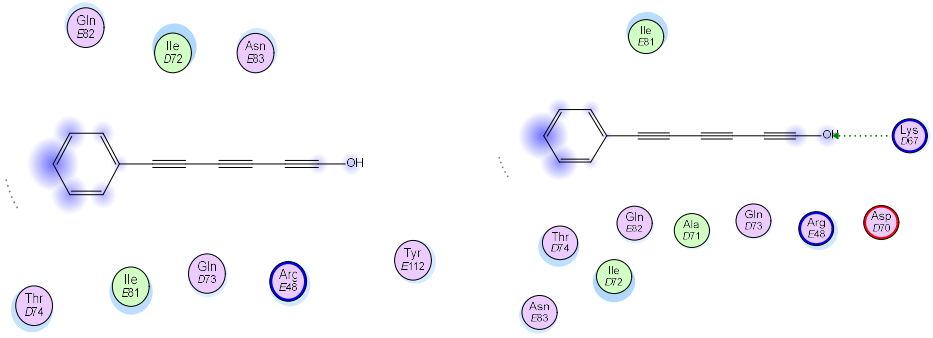
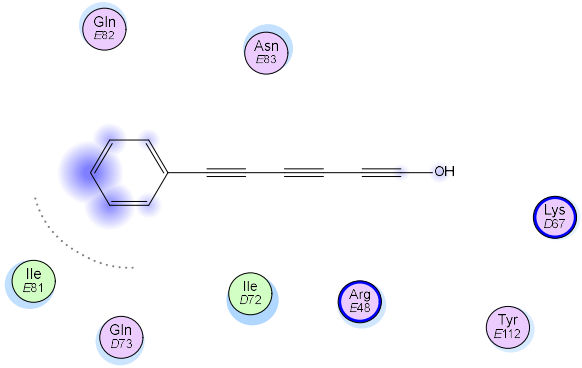
Gly E47
Ile D72
Lys D67
Ala D71
Asp D70

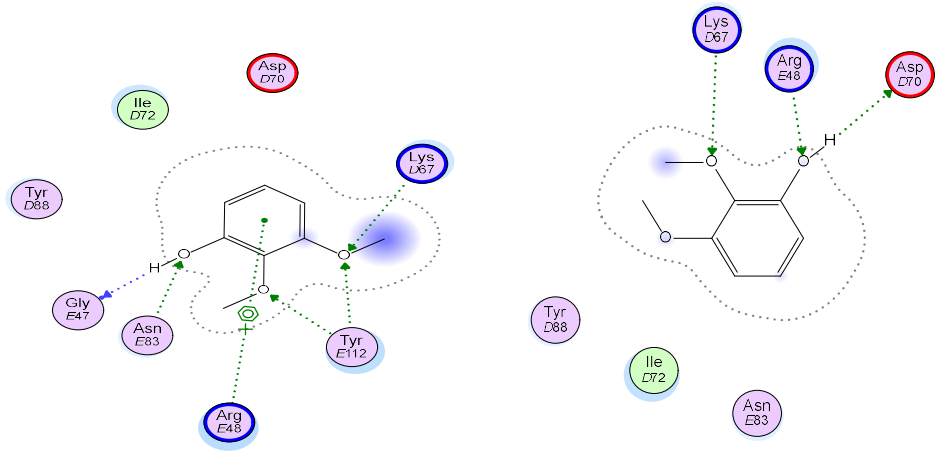


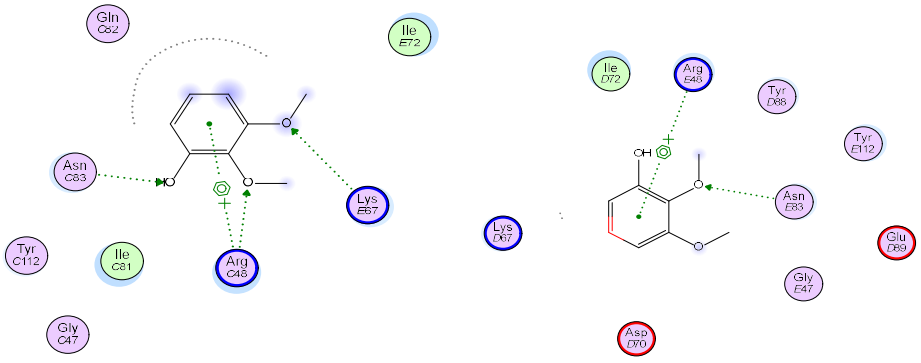
Lingand interactions for compound 7-Phenyl-hepta-2,4,6-triyn-2-ol with TNF

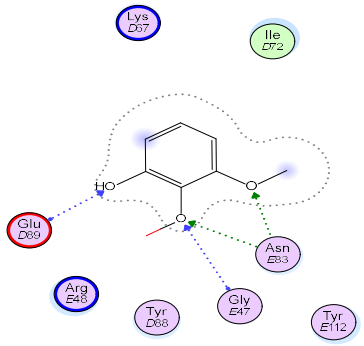




Lingand interactions for compound 20 with TNF





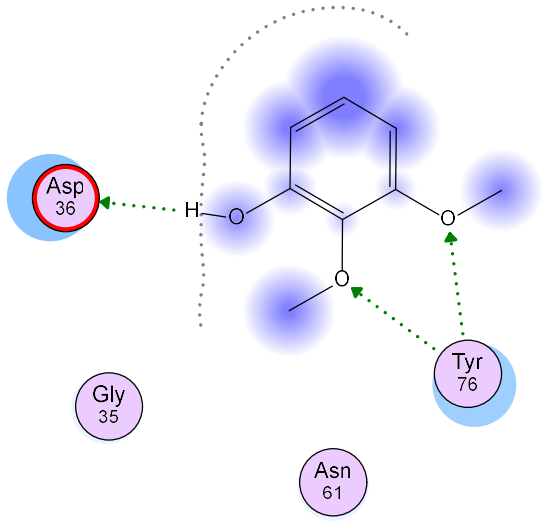


RESULTS FROM 1T1B TARGET WITH COMPONENTS IN BIDENS PILOSA

COMPOUND 20

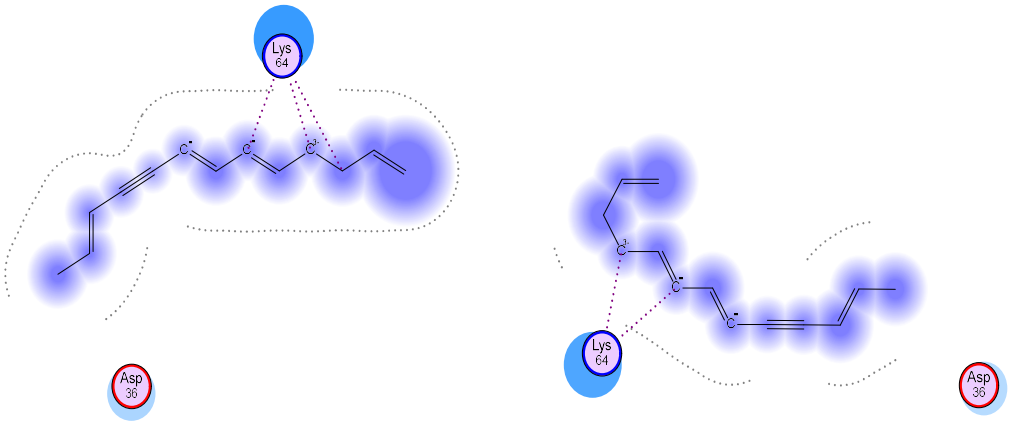
COMPOUND 20	S	E SCORE	ENERGY
143	-10.0825	-9.4511	-5.8223

Lingand interactions for compound 20 with 1T1B



Lingand interactions for compound Trideca-1,11-diene-3,5,7,9-tetrayne with 1T1B

COMPOUND	S	E SCORE	E REFINE
155	-12.8749	-7.6673	-14.9873
156	-12.3217	-8.7721	-37.1082



RESULTS FROM PTGS2 TARGET WITH COMPONENTS IN BIDENS PILOSA

Lingand interactions for compound Trideca-1,11-diene-3,5,7,9-tetrayne with PTGS2

COMPOUND 22

COMPOUND	S	E SCORE	E REFINE
152	-17.3659	-15.6284	114.6315
153	-17.2929	-14.7868	113.2064
154	-17.2664	-14.7414	74.5687
155	-17.1193	-15.2946	108.8418

COMPOUND 8

51	**-12.3942**	**-13.0077**	**7.7106**
52	**-12.3612**	**-13.3731**	**8.7525**
53	**-11.5789**	**-14.3171**	**-2.1945**
54	**-11.4540**	**-14.3328**	**8.7664**
55	**-11.2983**	**-13.0550**	**12.1880**
56	**-11.0594**	**-13.4297**	**-4.1645**
57	**-10.6921**	**-12.9634**	**-5.7177**

COMPOUND 20

140	**-13.5761**	**-13.2977**	**14.0134**
141	**-12.3187**	**-12.1450**	**7.5697**
142	**-11.2900**	**-12.2041**	**9.1230**
143	**-10.3420**	**-12.6033**	**28.7931**
144	**-10.3164**	**-12.2843**	**4.8871**

COMPOUND 47

364	**-14.3471**	**-13.4628**	**13.6517**
365	**-13.4950**	**-12.2697**	**16.6527**
366	**-11.8759**	**-14.0551**	**-1.2268**

367	**-10.5668**	**-12.6891**	**19.0957**
368	**-11.1957**	**-12.3843**	**2.8167**
369	**-10.5668**	**-12.7633**	**-0.2213**

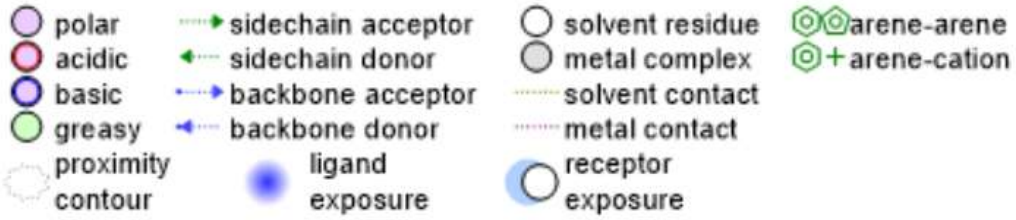
COMPOUND 24

161	**-10.6430**	**-9.4417**	**-1.4072**

COMPOUND 46

356	**-10.9374**	**-10.8449**	**48.2119**
357	**-10.4545**	**-11.5320**	**39.3753**
368	**-10.3735**	**-11.4535**	**5.4771**
359	**-10.2881**	**-10.8582**	**11.5894**

The figure 3 below shows different interactions of the protein and compounds



CONCLUSION

E score 1: Energy score 1. A numerical value representing the binding affinity or energy of interaction between the ligand and protein, calculated using a specific scoring function (e.g., London dG, Affinity dG).

E refine: Energy refinement. A process that optimizes the ligand's position and conformation within the binding site to improve the fit and minimize energy.

Basing on the above parameters these are the compounds with favourable anti-malaria effects towards malaria parasites ie, Compound 22, 8,47 and compound 24 are the favourable phytochemicals from bidens pilosa towards the treatment of malaria basing on the results from molecular operating environment during the docking process as indicated in the tables and ligand interactions in chapter 3

RECOMMENDATIONS

1. The phytochemicals were not tested on human beings therefore more research is required to establish and try out these on humans through extraction of the phytochemicals.
2. Though the research was done but more studies need to b done on other plants to establish the mode of action of different phytochemicals towards the treatment of malaria

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