ANTIMALARIA EFFECTS OF ARTEMESIA SPICE USED IN ANIMAL PRODUCTS FOR THE DEVELOPMENT OF FUNCTIONAL FOODS

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ABSTRACT
The study evaluated the docking scores, LD50 values, toxicity classes, amino acid affinities, bonds, and charges of artemesia compounds with crystal structure of Plasmodium falciparum dihydroorotate dehydrogenase (7KZ4). An in-silico approach was employed to assess the interactions between compounds present in artemesia and molecular targets implicated which was sourced from ethnobotanical databases, were prepared using the Ligprep module for molecular docking. Among the fifteen compounds analyzed, Isovitexin demonstrated the highest binding affinity with a docking score of -9.539, followed by Trolox (-8.501) and Casticin (-8.22). The toxicity analysis revealed that most compounds fell into toxicity classes 4 and 5, indicating low to very low toxicity. Isovitexin, despite its superior binding affinity, showed moderate toxicity (class 3, LD50 159 mg/kg). Notable compounds with favorable safety profiles included Casticin and Crisiliniol, both exhibiting LD50 values of 5000 mg/kg (class 5), while maintaining good binding scores of -8.22 and -7.472, respectively. Reference compounds such as Chloroquine and Artemisinin showed moderate binding affinities (-4.474 and -6.353, respectively) with varying toxicity profiles. These findings reveal that compounds like Casticin and Trolox may offer promising leads for further development, given their combination of strong binding affinities and favorable toxicity profiles.
Keywords: Antimalaria, Artemesia, Spice, Animal Products and Functional Foods

INTRODUCTION
The Food and Agriculture Organization (FAO) defined functional foods as foods that contain, in addition to nutrients, other components that may be beneficial to health. Similarly, the Mayo Clinic defines these foods as foods that have a potentially positive effect on health beyond basic nutrition" (Zeratsky, 2022). The Academy of Nutrition and Dietetics recently published a short article that discusses functional foods as food for wellness (Ellis E. Functional Foods 2022).

It has been known for a long time that a clear relationship exists between the food we eat and our health. When we eat food, our first and most basic aim is to obtain nutrients for our body and to satisfy our metabolic needs. However, some groups of foods, in addition to their nutritional properties, present other additional properties for health. These types of foods are called functional foods and may be defined as any food that has a positive impact on an individual's health, physical performance, or state of mind, in addition to its nutritious value. Other additional considerations have been proposed to define a functional food, including three additional conditions in particular:1. it is a food (not a capsule, tablet, or powder) derived from natural ingredients; 2. it can and should be consumed as part of the daily diet; 3. it has a particular function when eaten, serving to regulate a particular body process, - enhancement of biological defence mechanisms, prevention of specific diseases, recovery from specific diseases, control of physical and mental disorders and slowing of the aging process.

Herbs and spices have been used to fortify foods throughout history as preservatives, flavour and therapeutic agents. Although herbs and spices are low-cost commodities, they are nowadays valued as gold or jewels for many centuries. Herbs and spices were used by the ancient Egyptians and have been used for Centuries in India and China. Today, herbs and spices can be used to increase the acceptability of foodstuffs and improve their health. World Health Organization survey pointed that 70–80% of the world population depends on modern medicine mainly on herbal sources in their major healthcare (Chan, 2003). Moreover, 80% of population in developing Countries and up to 60% of the world's population depends directly on herbs and plants for their medical benefits (Shrestha and Dhillon, 2003).
Malaria is a parasitic infection transmitted by the Anopheles mosquito that leads to acute life-threatening disease and poses a significant global health threat (WHO, 2023). In 2022, there were 249 million malaria cases worldwide, with approximately 3.2 billion people at risk of malaria infection across 85-87 endemic countries (WHO, 2023). The Plasmodium parasite has a multistage lifecycle, which leads to characteristic cyclical fevers (Ashley *et al.,* 2018). With timely treatment, most people experience rapid resolution of symptoms; however, significant complications may occur, including cerebral malaria, severe malarial anemia, coma, or death (Ashley et al., 2018).

In endemic regions, malaria continues to be a major public health concern, with an estimated 608,000 deaths reported in 2022 (WHO, 2023).
Therefore, this study evaluates the functionality and antimalaria effect of *artemesia annua* in animal products.

MATERIALS AND METHODS

Materials: Computer (laptop), Schrödinger Suite 2023 13.5, Web servers (Protox-II) and selected bioactive compound of artemesia which was obtained from Pubchem site (https://pubchem.ncbi.nlm.nih.gov)
Ligand Preparation: The Ligprep module from Schrödinger Suite 2023 13.5 was utilized to prepare a total of 15 phytocompounds extracted from artemesia, as identified from ethnobotanical databases in preparation for molecular docking.
Protein Preparation: The Crystal structure of Plasmodium falciparum dihydroorotate dehydrogenase (7KZ4). X-RAY diffraction, with a resolution 1.75 Å, R-Value free 0.206, R-Value work 0.172 and R-Value observed 0.173.
Receptor Grid Generation: Using the receptor grid construction tool in Schrödinger Maestro 13.5, the scoring grid was defined and supported by the co-crystalized ligand GK.
Molecular Docking: Molecular docking of the prepared ligands and proteins were performed using the glide ligand docking in Schrödinger Suite 2023.

RESULTS

Table 1 shows the docking score, LD50 and toxicity class. Chloroquine has a moderate docking score of -4.474 and an LD50 of 750 mg/kg, placing it in toxicity class 4. Trolox has a good docking score of -8.501 but a relatively low toxicity class 4 with an LD50 of 1630 mg/kg. Germacrene has a docking score of -5.722 and a high toxicity class 5 with an LD50 of 5300 mg/kg. .

Table 1: The docking score, LD50 and toxicity class

Ligand	Compound CID	Docking score	LD50 (mg/kg)	Toxicity class
Chloroquine	2719	-4.474	750	4
Isovitexin	162350	-9.539	159	3
Trolox	40634	-8.501	1630	4
Casticin	5315263	-8.22	5000	5
Eupatorin	97214	-7.996	4000	5
Crisilineol	162464	-7.472	5000	5
Artemisin	65030	-6.353	900	4
Scopletin	5280460	-6.303	3800	5
Germacrene	5317570	-5.722	5300	5
Artemisinin	68827	-5.358	4228	5
Alpha-Terpinene	7461	-5.09	2500	5
"(5r)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene"	2723720	-4.861	3700	5
Myrtenol	10582	-4.854	2100	5
Alpha -humulene	5281520	-4.808	3650	5

Alpha-humulene has a docking score of -4.808 and a toxicity class 5 with an LD50 of 3650 mg/kg. Myrtenol has a docking score of -4.854 and a toxicity class 5 with an LD50 of 2100 mg/kg. Artemisin (CID 65030) has a docking score of -6.353 and an LD50 of 900 mg/kg, placing it in toxicity class 4. Scopletin (CID 5280460) has a docking score of -6.303 and a high toxicity class 5 with an LD50 of 3800 mg/kg. Alpha-Terpinene (CID 7461) has a docking score of -5.09 and a toxicity class 5 with an LD50 of 2500 mg/kg. (5r)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene has a docking score of -4.861 and a high toxicity class 5 with an LD50 of 3700 mg/kg. Isovitexin (Compound CID: 162350) has the highest binding affinity with a docking score of -9.539, and it falls into toxicity class 3 with an LD50 of 159 mg/kg, indicating moderate toxicity. Trolox (CID: 40634) also shows strong binding with a docking score of -8.501 and has an LD50 of 1630 mg/kg, placing it in toxicity class 4, which is slightly toxic. Casticin (CID: 5315263) exhibits strong binding with a docking score of -8.22 and is practically non-toxic with an LD50 of 5000 mg/kg, classified in toxicity class 5. Eupatorin (CID: 97214) has a good docking score of -7.996 and an LD50 of 4000 mg/kg, indicating very low toxicity (class 5). Similarly, Crisiliniol (CID: 162464) has a docking score of -7.472 and an LD50 of 5000 mg/kg, also in toxicity class 5. Artemisinin (CID: 68827) has a docking score of -5.358 and an LD50 of 4228 mg/kg, indicating very low toxicity (class 5).

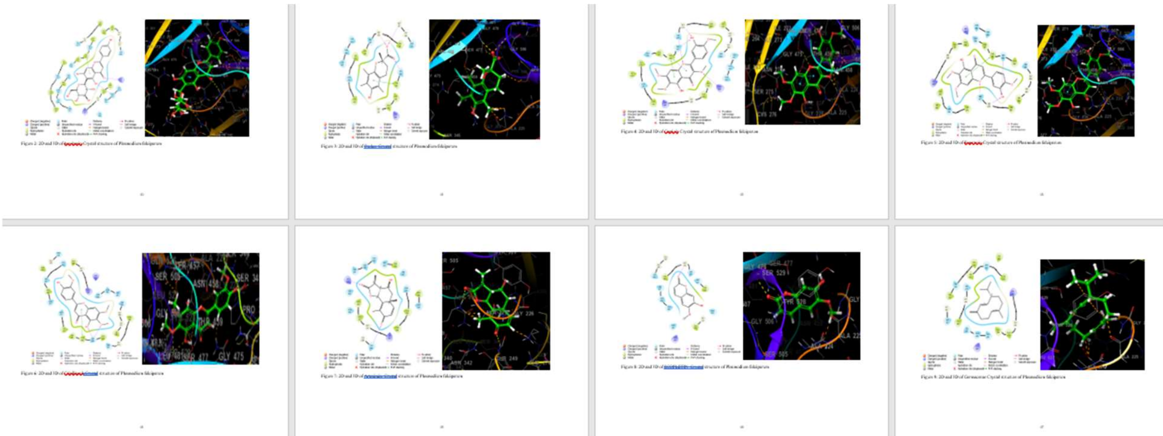

Figure1: 2D and 3D of Artemesia compoumd with Crystal structure of Plasmodium falciparum

DISCUSSION

Chloroquine is a well-known antimalarial drug with a docking score of -4.474 and an LD50 of 750 mg/kg (Morales-Bayuelo *et al*., 2024 and Joseph *et al.,* 2020). It has been widely used for the treatment and prevention of malaria infections caused by Plasmodium falciparum and other species (Antimalarial ligands, 2024).

Isovitexin has the highest docking score of -9.539 among the listed compounds, indicating strong binding affinity to potential malarial targets (Morales-Bayuelo *et al*., 2024). However, its relatively low LD50 of 159 mg/kg suggests higher toxicity compared to other compounds (Joseph *et al.,* 2020). Trolox has a docking score of -8.501 and an LD50 of 1630 mg/kg, suggesting potential antimalarial activity with a balance between efficacy and safety (Morales-Bayuelo *et al*., 2024 and Joseph *et al.,* 2020). Casticin, Eupatorin, Crisilineol, Artemisinin, and other compounds with toxicity class 5 exhibit docking scores ranging from -8.22 to -5.09 (Morales-Bayuelo *et al*., 2024). These compounds are unlikely to present acute hazards in normal use and may serve as potential leads for antimalarial drug development (Joseph *et al.,* 2020).

CONCLUSION

These findings reveal that compounds like Casticin and Trolox may offer promising leads for further development, given their combination of strong binding affinities and favorable toxicity profiles.

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