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# Tryparedoxin peroxidase of *Leishmania braziliensis*: homology modeling and inhibitory effects of flavonoids for anti-leishmanial activity

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**Abstract:**
Inhibition of the Tryparedoxin peroxidase interaction has been becomes a new therapeutic strategy in leishmaniasis. Docking analysis was carried out to study the effects of quercetin and taxifolin on Tryparedoxin Peroxidase (TryP). Tryparedoxin peroxidase of Trypanosomatidae functions as antioxidants through their Peroxidase and peroxynitrite reductase activities. The 3D models of Tryparedoxin Peroxidase of *Leishmania braziliensis* (*L. braziliensis* TryP) was modeled using the template Tryparedoxin Peroxidase I from *Leishmania Major* (*L. Major* TryPI) (PDB ID: 3TUE). Further, we evaluated for TryP inhibitory activity of flavonoids such as quercetin and taxifolin using *in silico* docking studies. Docking results showed the binding energies of -11.8601and -8.0851 for that quercetin and taxifolin respectively. Flavonoids contributed better *L. braziliensis* TryP inhibitory activity because of its structural parameters. Thus, from our *in silico* studies we identify that quercetin and taxifolin posses anti-leishmanial acitivities mediated through TryP inhibition mechanism.

**Keywords:** *Leishmania braziliensis*, Tryparedoxin Peroxidase, homology modeling, Quercetin, Taxifolin.

**Background:**
Leishmaniasis is a major health problem that affects approximately 12 million people worldwide with 2 million new cases diagnosed every year. The precarious life conditions, the social disorganization and the lack of an effective political action and educational programs contribute to persistence of these diseases in the poorest regions of the world. Malaria and leishmaniasis are the most prevalent neglected diseases caused by protozoan parasites. Half of world's population is at risk of malaria. More than 500 million of people become severely ill with nearly a million people die due to plasmodium infection every year. Currently, leishmaniasis threatens 350 million of people around the world; more than 2 million of new cases of leishmaniasis occur annually (http://www. who.int/tdr/ diseases/leish/diseaseinfo.htm). Brazil, India, Bangladesh and Sudan present 90% of cases of visceral leishmaniasis around the world. According to WHO, in the last two decades the number of cases of leishmaniasis increased due to the widening of endemic areas and the emergence of new focus of leishmaniasis. Epidemiological studies indicate that deforesting, unsettled growth of urban centers and changing habits of the insect vector are contributing to disease urbanization and new endemic foci **[1 2, 3]**. The causative agents of this disease are parasites of the genus *Leishmania*, which infect and replicate in macrophages of the vertebrate host. Leishmaniasis presents a broad clinical spectrum, ranging from asymptomatic and self healing infections to those causing significant mortality **[4]**. The parasite completes its life cycle in two hosts, namely sand fly and humans **[5]**. The organism is found in approximately 90 countries around the world, including Tropical Africa, South America, Central and East Asia, and Southern Europe. This disease is endemic in low-income population of Central and South American countries **[6]**. Which is caused by over twenty one different species of

 

*Leishmania* parasites (e.g. *L. donovoni*, *L. infantum*, *L. braziliensis, L. major*, *L. mexicana*, *etc.).* The parasite has a digenetic life cycle alternating between the promastigote forms in the sand fly insect, and the amastigotes within the mammalian host. Once inside the macrophage phagolysosomes, the promastigote-to-amastigote differentiation is triggered through activation of several regulatory mechanisms **[7-9]** There are mainly three forms of the disease namely, cutaneous (self healing skin ulcer) (e.g. *L. major*) to mucocutaneous (e.g. *L. braziliensis*) to visceral (e.g. *L. donovoni*, *L. infantum*) out of which cutaneous Leishmaniasis is the most common while visceral Leishmaniasis is the lethal form. The letter is invariably fatal if left untreated. Current treatments are unsatisfactory, side effects; high cost and low efficacy better drugs are urgently required **[10]**. Most of the parasites, including *Leishmania Spp*., are more susceptible to reactive oxygen species than their hosts **[11, 12]**.

Till date, there has been no effective vaccine against leishmaniasis and the treatment relies exclusively on chemotherapy. Pentavalent antimonials have been the mainstay of therapy for all forms of leishmaniasis for last seven decades, however, its efficacy has declined in recent years with the result that only about one third of patients respond to it **[13, 14]**. Tryparedoxin Peroxidase belongs to the protein family of peroxiredoxins **[7-9]**. This enzyme cascade involves trypanothione reductase (Try R), tryparedoxin (TXN) and trypanotione ($N^1$, $N^8$-bis (glutathionyl)-spermidine) serving as a mediator for transfer of reducing equivalents **[15-17]**. The first enzyme of the cascade is homologous to glutathione reductase and thioredoxin reductase **[16]** which are involved in NADPH-dependent hydroperoxide reduction in other species **[18]**. The other components of the trypanosomatid system also belong to protein families occasionally constituting peroxidase systems. Preliminary amino acid sequencing data indicated that tryparedoxin is phylogenetically related to thioredoxin, whereas the tryparedoxin peroxidase belongs to the peroxiredoxins **[19]** comprising the thioredoxin peroxidases of yeast and mammals **[20]** and the alkyl hydroperoxide reductases of bacteria **[21]**.

The present possibilities available for the treatment of trypanosomal diseases, such as Chagas disease, African sleeping sickness, and the various forms of leishmaniasis, necessitate improvement. We like many others have therefore embarked on the identification and characterization of potential molecular targets typical of the trypanosomatids. The natural product quercetin and taxifolin is a flavonoids found in many fruits and vegetables. Previous research has shown that quercetin and taxifolin has antitumor, anti-inflammatory, antiallergic, and antiviral activities. Taxifolin/ Dihydroquercetin a dihydroflavonol belongs to flavonoids group, together with its glycosides are commonly found in many species of medical plants. Dihydroquercetin is the most powerful natural antioxidant. Different studies show that it has hypocholesterolemic effects, and also demonstrates anti-inflammatory activities **[22]** anti-acne activity **[23]**, medical applications include but not limited to: vitamin deficiency as a vitamin P, cure atherosclerosis, poison treatment, inhibit the cancer cells development, Helps in recovery after chemotherapy and radiation treatment, chemopreventive activity **[24]**. Fights the chronic fatigue syndrome and metabolic syndrome. Dihydroquercetin offers protection against cardiovascular disease by inhibiting several steps in the disease process. Additionally, dihydroquercetin helps guard nervous system health, prevents the complications of diabetes, protects the liver against hepatitis-inducing agents, fights infection, and quells inflammation that can lead to dermatitis, arthritis, and pain.

Quercetin has a synergistic effect with ephedrine and caffeine, increasing and prolonging their properties. Quercetin acts as a potent antioxidant and inhibits inflammatory and allergic reactions by inhibiting histamine release and other allergy-mediating compounds. Quercetin may also reduce capillary fragility, and it may offer protection against diabetic cataracts by inhibiting aldose reductase in the lens. Quercetin might reduce cancer risk by inactivating malignant precursors or by inhibiting carcinogenesis. Preliminary studies suggest it might have inhibitory effects on various cancer types, including breast, leukemia, colon, ovary, oral squamous cell, endometrial, gastric and non-small-cell lung carcinomas. Quercetin has antiestrogenic effects in cultures of breast cancer cells. Quercetin may be beneficial in benign prostatic hyperplasia (BPH), alopecia, hirsutism, androgen-dependent disorders, bacterial prostatitis, prostate cancer, atherosclerosis, hypercholesterolemia, coronary heart disease, vascular insufficiency, diabetes, cataracts, allergies, allergic rhinitis, peptic ulcer, schizophrenia, inflammation, asthma, gout, viral infections such as herpes simplex virus and in preventing cancer.

The detailed *in silico* analyses of probable inhibition as well as interaction of the models were performed with high binding affinity. However there is no conclusive report as to whether the antileishmanial activity of the taxifolin and quercetin. In the present study, the structural models of the taxifolin and quercetin in the trypanothione reductase binding sites has been carried out, which may facilitate further development of more potent antileishmanial agents. Taxifolin and quercetin might be a promising additive in combined drug inhibitor of trypanothione reductase.

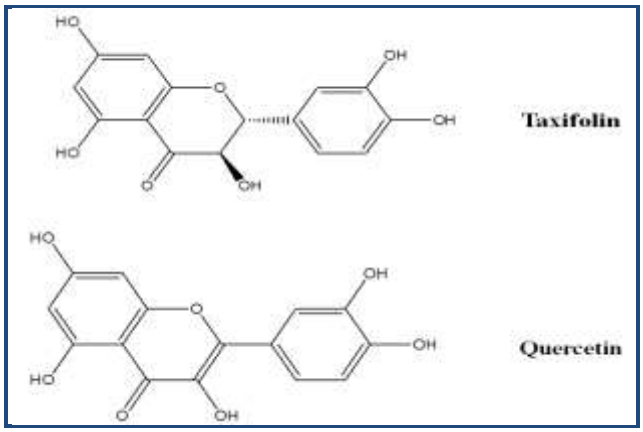


**Figure 1:** Structures of quercetin and taxifolin.

**Methodology:**

***Target and template Sequence alignments***

The sequence of *L. braziliensis* TryP (gi No. 154334618) was retrieved from National Centre for Biotechnology Information (NCBI). The homology sequences were searched against the

Protein Data Base **[25]** using NCBI-BlastP **[26-27]**. The most homologous structure obtained was considered as the potential template for modeling the structure of target **[28]**. The atomic coordinates for modeling the structure was obtained from the protein databank.

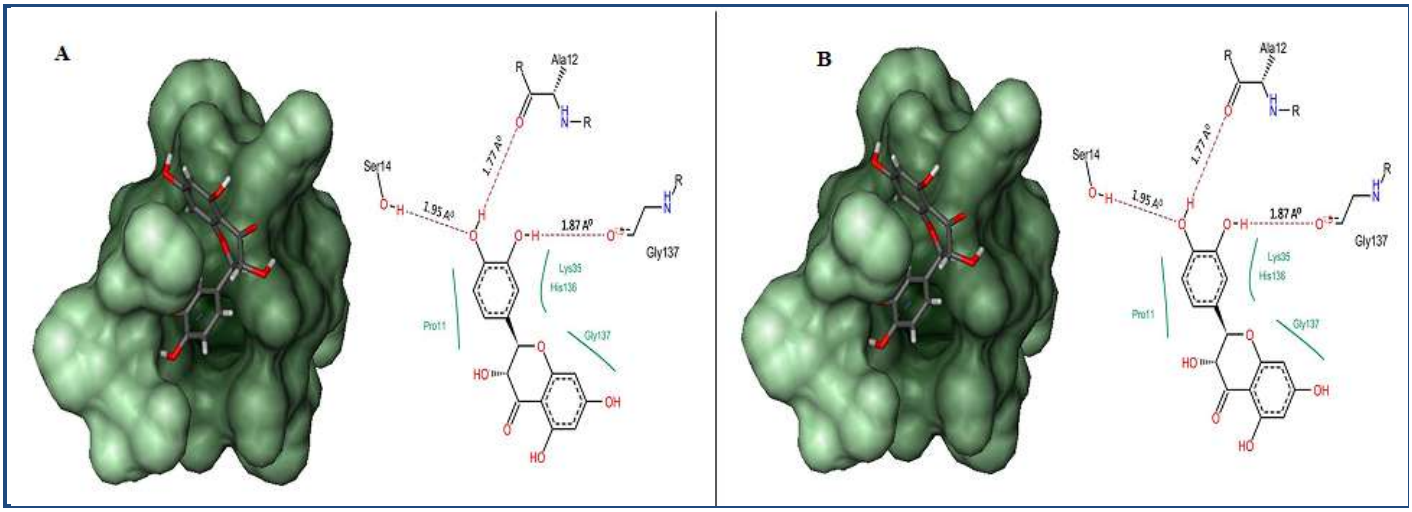


**Figure 2: A)** Interaction of taxifolin with Tryparedoxin Peroxidase (3TUE); **B)** Interaction of Quercetin with Tryparedoxin Peroxidase (3TUE).

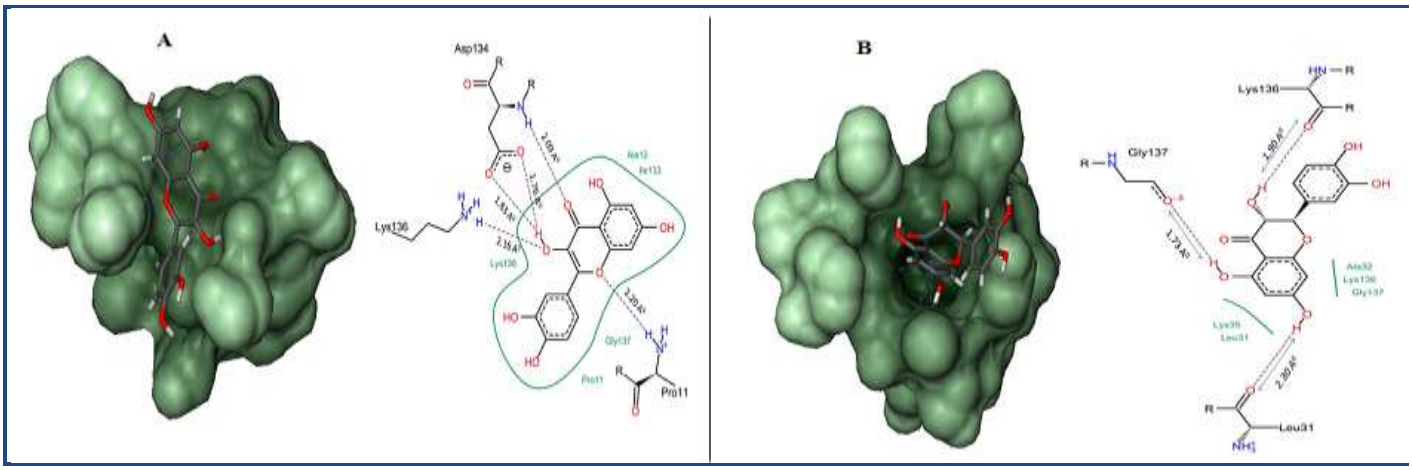


**Figure 3: A)** Interaction of Quercetin with Tryparedoxin Peroxidase from *L. braziliensis*; **B)** Interaction of taxifolin with Tryparedoxin Peroxidase from *L. braziliensis*.

***Prediction of binding site***

To determine the interactions between flavonoids (Quercetin and Taxifolin) and TryP the amino acids in the binding site of the model was predicted through Q-site Finder **[29]** and the same was confirmed by the conserved residues observed in the template binding site.

***Ligand preparation***

The flavonoids like Quercetin and Taxifolin (**Figure 1**) molecules were drawn in ACD-Chemsketch (www.acdlabs .com) and their SMILES notation was obtained. They were converted into SDF files using 'Online SMILES convertor and Structure file generator' **[30]**.

***Flexible docking***

Molecular docking analysis is carried out between the target protein active site with ligands of flavonoids like Quercetin and Taxifolin. The developed SDF structures were docked within the binding site of *L. braziliensis* Try P using FlexX **[31]** with following parameters i) default general docking informations, ii) base placement using triangle matching, iii) scoring of full score contribution and threshold of 0, 30 and No score contribution and threshold of 0,70. iv) Chemical parameters of clash handling values for protein ligand clashes with maximum allowed overlap volume of 2.9 $A^{03}$ and intra-ligand clashes with clash factor of 0.6 and considering the hydrogen in internal clash tests. v) Default docking details values of 200 for both the maximum number of solutions per iteration and maximum number of solutions per fragmentation.

***Predictions of ligand-receptor interaction***

The interaction of flavonoids with *L. braziliensis* Try P in the docked complex were analyzed by the pose-view of LeadIT **[32]**.

**Results & Discussion:**

***Docking studies with flavonoids***

These quercetin and taxifolin-two flavonoids were docked with modeled *L. braziliensis* Try P and also with its template *L. Major*

Try PI using the docking program FlexX and the ligand-receptor interactions were analyzed using LeadIT. The docking interactions of the quercetin and taxifolin molecules with modeled *L. braziliensis* Try P and its template *L. Major* Try PI implies that the taxifolin has lowest binding energy of -8.0851 kJ $mol^{-1}$ and -7.5118 kJ $mol^{-1}$ respectively and quercetin exhibited highest binding energy of -11.9518 kJ $mol^{-1}$ and -9.2482 kJ $mol^{-1}$ respectively. This observation suggests that the folding of *L. braziliensis* Try P would be the almost same as its template protein *L. Major* Try PI and **Table 1 (see supplementary material)** summarizes these results. **Figure 2** & **3** show the docking results of modeled *L. braziliensis* Try P and its template *L. Major* Try PI proteins **Table 2 (see supplementary material)** shows the corresponding amino acids with their specific binding energies favoring the interactions.

The binding energy scores of the taxifolin and quercetin molecules with *L. braziliensis* TryP -8.0851 kJ $mol^{-1}$ and -11.8601 kJ $mol^{-1}$ respectively and in the case of template *L. Major* Try PI -7.5118 kJ $mol^{-1}$ and -9.2482 kJ $mol^{-1}$ of binding energy score with taxifolin and quercetin respectively. The amino acids that interacted with quercetin were found to be Pro-11, Asp-134 and taxifolin were found to be Ala-12, Ser-14, Gly-137 in *L. Major* Try PI **(Figure 2a & b)** and Pro-11,Asp-134, Lys-136 in *L. braziliensis* Try P with quercetin and Leu-31, Lys-136, Gly-137 in *L. braziliensis* Try P with taxifolin (**Figure 3a & b).** These findings suggested that the amino acids Proline (Pro), Aspartic acid (Asp), Glycine (Gly) and Lysine (Lys) in the active site of Try P of template and modeled proteins were conserved and favoring the interactions with the ligands.

**Conclusion:**

In conclusion, this study clearly indicates that quercetin and taxifolin have excellent binding interactions with *L. braziliensis* Try P and *L. Major* Try PI. The docking results indicated that the amino acid residue Lys136 seemed to be essential in *L. braziliensis* Try P ligand recognition through a critical hydrogen bonding interaction with the docked ligands. Our ongoing studies on identification of novel and specific inhibitors of these generated homology model is expected to be useful for the structure based drug design against leishmaniasis.

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## Supplementary material:

**Table 1:** Binding energies of docked flavanoids with the modeled protein *L. braziliensis* and its template TryPI from *L. Major*.

| Receptors | Ligands with binding energies (kJ/mol) | |
|---|---|---|
| | Taxifolin | Quercetin |
| | -8.0851 | -11.8601 |
| *braziliensis* TryP (predicted model) | | |
| *L. Major* TryPI (3TUE) template | -7.5118 | -9.2482 |

**Table 2:** Binding site residues and their type of interactions in *L. braziliensis* and its template TryPI from *L. Major*

| Protein | Template Protein | | Modeled Protein | |
|---|---|---|---|---|
| Ligands | Taxifolin | Quercetin | Taxifolin | Quercetin |
| **Amino acids involved in bonded interactions** | | | | |
| | - | Pro11 | - | Pro 11 |
| | Ala 12 | - | - | - |
| | Ser 14 | - | - | - |
| | - | - | Leu 31 | - |
| | - | - | - | - |
| | - | Asp 134 | - | Asp 134 |
| | - | - | Lys 136 | Lys 136 |
| | Gly 137 | - | Gly 137 | - |
| **Amino acids involved in bonded interactions** | | | | |
| | Pro11 | Pro11 | - | Pro11 |
| | - | Ala12 | - | Ala12 |
| | - | - | Leu31 | - |
| | - | - | Ala32 | - |
| | Lys35 | - | Lys35 | - |
| | - | Ile133 | - | Ile 133 |
| | - | - | - | - |
| | His136 | - | Lys136 | Lys136 |
| | Gly137 | Gly137 | Gly137 | Gly137 |
| **Docking score(kJ/mol)** | -7.5118 | -9.2482 | -8.0851 | -11.8601 |

 