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Research Article

In-Vitro Leishmanicidal Activity and Molecular Docking Simulations of a Flavonoid Isolated from *Pistacia integerrima* Stew ex Brandis

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Pistacia integerrima Stew ex Brandis is a valued medicinal plant used for curing various diseases such as diarrhea, fever, liver disorder, pain, asthma, and inflammation. The aim of this study was the isolation of bioactive leishmanicidal agents from the methanolic extract. The methanolic extract led to the isolation of flavonoids 3,5,7,4′-tetrahydroxy-flavanone (1). The extract and isolated compound 1 were tested for antileishmanial effect. The extract showed a percent effect of 63.09 with an IC_{50} value (49.32 μM). The isolated compound 1 was more leishmanicidal than the extract with a percent growth inhibition of 68.09. We have performed docking studies on two antileishmanial targets; homology modeled dihydrofolate reductase (DHFR) and pteridine reductase (PTR1) from *Leishmania major* (*L. major*). Interaction with important residues of the studied enzymes revealed the possible mechanism of in-vitro activity against promastigotes of *L. major*.

1. Introduction

Pistacia integerrima is an important medicinal plant belonging to the family Anacardiaceae. It is commonly known as a crab's claw or Zebrawood. It is native to Asia and distributed in various countries including Afghanistan, India, and Pakistan and grows at a high altitude of 800–1900 meter [1]. Since early times, various parts of *Pistacia integerrima* are used in the preparation of herbal medicine [2]. In traditional systems particularly, its galls have been used for the treatment of renal disorder, dysentery, asthma, appetite, phthisis, cough, dyspeptic vomiting, and snakebite [3]. *Pistacia integerrima* is being used for curing respiratory tract and gastrointestinal disorders [4, 5]. *Pistacia*

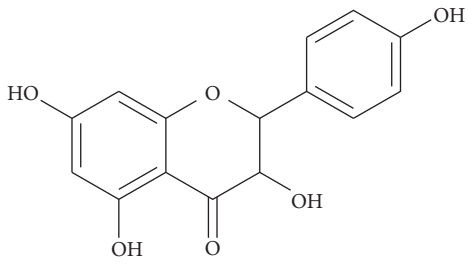


Figure 1: Chemical structure of 3,5,7,4′-tetrahydroxy-flavanone (1) isolated from *P. integerrima*.

integerrima extract has been reported for various biological efficiency such as anti-inflammatory, analgesic, muscle relaxant, gastrointestinal, and antiemetic properties [6–9]. *P. integerrima* different parts have been explored phytochemically and various classes of compounds such as sterols, triterpenoids, tannins, flavonoids, etc. are reported [10–12]. The isolated compounds have been reported for anti-inflammatory, analgesic, muscle relaxant, sedative, and antipyretic activity [13].

The leishmaniasis and its outspread in some developing countries are challenging the health system. Various research groups worked on different strategies to overcome the resistance and side effects of the conventional antileishmanial drugs, by targeting trypanothione reductase of tissue in the host cell [14] via a synthetic analog of ferrocenylquinoline [15], the evaluation of chromone derivatives in visceral leishmaniasis [16], and application of fractions of mushroom (*Grifola frondosa*) [17]. Based on the abovementioned literature, targeting the common therapeutic targets among the various strains of leishmania will have probably more effects with the least adverse effects. Therefore, the aim of the present study is to evaluate the antileishmanial properties of *Pistacia integerrima* extract and the isolation of targeted phytochemicals responsible for the antileishmanial effect.

2. Material and Methods

2.1. Plant Collection. Pistacia integerrima galls were obtained from the mountain area of District Dir (L), KP, Pakistan. The plant specimen was identified by an expert plant taxonomist, at the University of Swabi, KP, Pakistan. The identified plant specimen was assigned voucher specimen number No. UOS/Bot-102. The specimen was stored in the herbarium of the Bantay Department, University of Swabi, KPK, Pakistan.

2.2. Extraction and Isolation. The galls were dried under shade and then washed with water to remove the dust. The shade-dried galls (3.00 kg) were subjected to hot extraction with help of the soxhlet apparatus. The extraction process was performed in triplicate to obtain the maximum extract. The obtained extract was filtered and then concentrated at low pressure and temperature by using a rotary evaporator which yielded crude extract (93.76 g/3.00 kg of galls). The methanolic extract was defatted with hexane to remove fatty acids and dyes. The defatted extract was assessed to normal phase Column chromatographic analysis, the column was eluted with a mixture of chloroform and hexane (95 : 5) which yielded compound 1. The structure of compound 1 was determined by comparing the physical and spectroscopic data with previously reported data [12, 18] (Figure 1).

2.3. Leishmanicidal Activity. The leishmanial species (*Leishmania major*) was cultured at the recommended temperature (25 ± 2.0) and standard conditions in RMPI-1640 (sigma) as per reported methods [19, 20]. The medium used was enriched with 10% heat-inactivated fetal bovine serum. In the logarithmic growth of promastigote, the culture was centrifuged at the speed of 2000 rpm for recommended duration and washed out with saline. The parasites were diluted with the fresh medium until the final density of 10^6 cells/ml was achieved. The microplate of 96 wells was used for this assay as the first row was supplemented with 180 ml of medium and 100 ml was added to the rest of the wells. The sample to be tested was diluted and added to the medium. The parasite culture (100 ml) was added to all wells. One of the wells was declared as negative control and was supplemented with DMSO while in the positive control well amphotericin B was added. The microplate was inoculated in standard laboratory conditions for 72 h. The ratio of survival of the parasite was counted. The result was taken in triplicate and the percent, as well as IC_{50}, was calculated.

2.4. Docking Studies

2.4.1. Docking Studies Using MOE Software. Molecular operating environment software (MOE 2016.0802) was used to perform docking simulations on dihydrofolate reductase from *L. major* (*Lm*DHFR) and pteridine reductase from *L. major* (*Lm*PTR1). For *Lm*DHFR, we performed docking on our previously constructed homology model of *Lm*DHFR. While for *Lm*PTR1, a 3-D crystal structure was obtained from PDB with PDB ID =1E7W. Preparation of ligand and proteins was carried out and docking studies were carried out using our previously reported methods [21, 22]. Two-dimensional (2-D) and three-dimensional (3-D) plots were visualized by using MOE and Discovery Studio Visualizer.

2.4.2. Docking Studies Using Autodock Software. Docking studies were also carried out using the AutoDock 4.2 version. Prepared proteins and ligands were taken from MOE software. AutoDock Tools (ADT 1.5.6) were used for Gasteiger charges calculation. Default parameters were used for docking runs.

In the case of homology-modeled LmDHFR, the first blind docking was carried out. Then, substrate dihydrofolic acid (DHFA) and methotrexate (MTX), a competitive inhibitor of DHFR, was docked. The important key residues were determined, which were used to determine the active site pocket. For docking on LmPTR1 (PDB ID =1E7W), grid

Table 1: Leishmanicidal activity of the extract and isolated flavonoid (1) from *P. integerrima*.

Tested sample	Concentration	Percent effect	IC_{50} (μM)
Extract	10 μg/mL	63.09 ± 1.54	49.32 ± 0.43
Compound 1	5 μg/mL	68.09 ± 0.76	30.43 ± 0.32
Amphotericin B	2.5 μg/mL	84.09 ± 0.40	0.22 ± 0.09

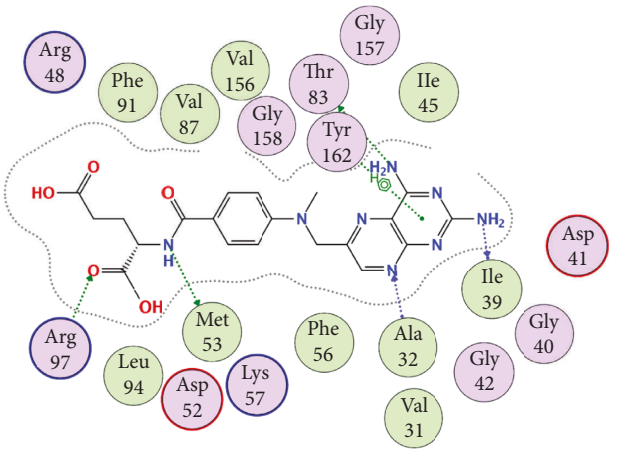


Figure 2: 2-D interaction plots of methotrexate into the active site of homology modeled *Lm*DHFR.

dimensions 60 × 60 × 60 with grid spacing 0.375 Å and *X*, Y, and *Z* coordinates fixed at 22.4, 3.67, and 12.79 respectively, were used. Discovery Studio Visualizer was used for visualization.

3. Results

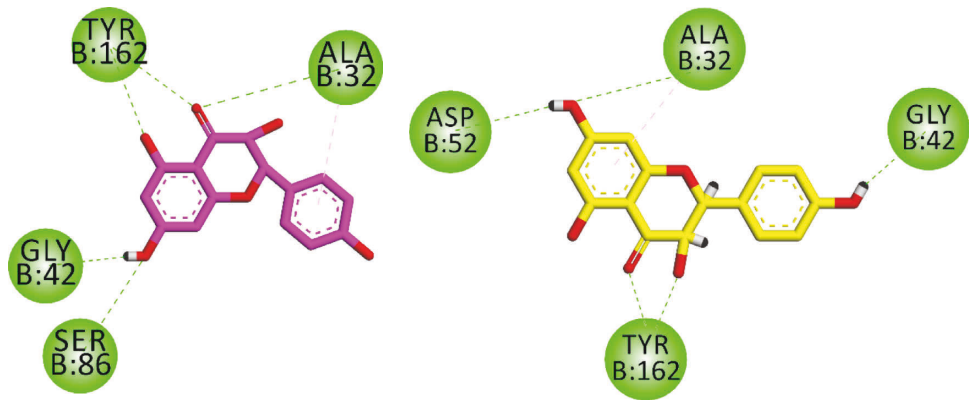
3.1. Antileishmanial Effect. The extract and isolated compound 1 were tested for antileishmanial effect. The extract showed a percent effect of 63.09 with an IC_{50} value (49.32 μM). The isolated compound 1 was more leishmanicidal than the extract with a percent growth inhibition of 68.09. The result of positive control (amphotericin B) was the maximum among the tested samples (Table 1).

3.2. Docking Studies. We have performed docking studies on two antileishmanial targets dihydrofolate reductase (DHFR) and pteridine reductase (PTR1). DHFR and PTR1 are considered key enzymes for the treatment of leishmaniasis. Molecular operating environment software was used to perform these studies. The three-dimensional crystal structure of DHFR from *L. major* is not available. Hence, we performed docking on our previously constructed homology model of *Lm*DHFR [21, 22]. Two-dimensional (2-D) interaction plot of clinically used methotrexate (MTX) showed that it established hydrogen bond interaction with Ala32, Ile39, Met53, Thr83, Arg97, and Tyr162 of *Lm*DHFR (Figure 2). While 2-D interaction plot obtained *via* MOE docking of a flavonoid isolated from *Pistacia integerrima* Stew ex Brandis established hydrogen bond interactions with Ala32, Gly42, Ser86, and a bifurcated hydrogen bond interaction with Tyr162 (Figure 3(a)). While Figure 3(b) represents the 2D interaction plot of the compound obtained through AutoDock. The isolated compound forms hydrogen bond interactions with Ala32, Gly42, Asp52, and Tyr162.

Next, we performed docking studies on PTR1 target (PDB ID = 1E7W). Our previous studies showed that MTX formed hydrogen bond interactions with Arg17, Lys198, and Gln186. Phe113 forms π-π stacking interaction with phenyl ring in the binding site of *Lm*PTR1 [22]. By using the MOE dock, the isolated compound forms hydrogen bond interactions with Gly13, Lys16, Arg17, Leu18, Ser111, and Asp181. Phe113 interacts with phenyl ring via π-π stacking interactions (Figure 4(a)). By using AutoDock, the isolated compound forms hydrogen bond interactions Arg17, Leu18, Asn109, Ser111, and Asp181. Phe113 forms π-π stacking interactions (Figure 4(b)).

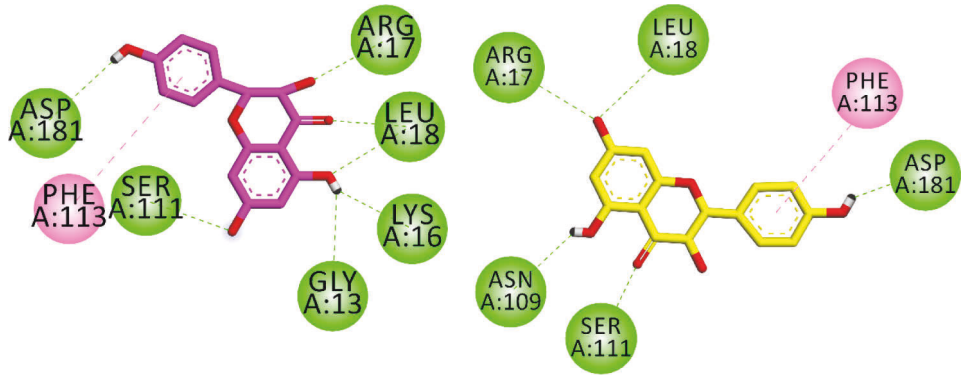
4. Discussion

The hidden potential of medicinal plants is needed to be discovered. These plant-based medicines are the treasure of multiple pharmacologically active molecules. In most cases, the medicinal plant is screened against the folklore but we encourage screening these medicinal plants against all possible activities [23]. In the current scientific modern era, the failure in therapy is due to poor compliance, and this poor compliance is the result of potential side effects of available drugs [24]. Currently, leishmania is treated with various drugs such as sodium antimony gluconate (SAG), amphotericin B, paromomycin, and miltefosine [24]. These antileishmanials (AL) are used as monotherapy or in combination [25]. In most cases, the AL is used in combination to reduce the risk of resistance [26, 27]. The combination therapy of AL is associated with various side effects, which are responsible for patient's poor compliance [28]. For the treatment of leishmania and to reduce the resistance as well as poor compliance, the discovery of safe, effective, and economical AL is the need of the day. Various research groups adopted diverse strategies for combating the resistance and side effects associated with conventional antileishmanial drugs. For instance, targeting trypanothione reductase of tissue in the host cell of leishmania [14] via a synthetic analog of ferrocenylquinoline [15], the evaluation of chromone derivatives in visceral leishmaniasis, which results from the activation of Th1 cell response [16], and application of fractions of mushroom (*Grifola rondose*) [17]. Based on the above-mentioned literature, targeting the common therapeutic targets among various strains of leishmania will have probably more effects with the least adverse effects. To discover the AL with the above-mentioned characteristics, the current project was designed. The *Pistacia integerrima* Stew ex Brandis is one of the potential



(a) (b)

Figure 3: 2-D interaction plots of isolated flavonoid obtained *via* (a) MOE dock (b) AutoDock into the active site of homology modeled *Lm*DHFR.



(a) (b)

Figure 4: 2-D interaction plots of isolated flavonoid obtained *via* (a) MOE dock (b) AutoDock into the active site of *Lm*PTR1 (PDB ID =1E7W).

medicinal plants having various pharmacological applications. The extract, as well as the isolated compound, was tested for the AL effect against L. major and both of the tested samples resulted significant AL effect. In the present study, in-vitro studies are carried out on promastigotes of L. major. Therefore, we carried out docking studies on two key antileishmanial target enzymes; dihydrofolate reductase (DHFR) and pteridine reductase (PTR1). The docking studies were carried out by using MOE and AutoDock software packages. These comparative docking studies showed the ligand forms interact with almost the same amino acid residues. Interaction plots of the isolated flavonoid in the binding site of enzymes revealed that *in-vitro* activity may be due to the inhibition of these two key enzymes.

5. Conclusions

Several drugs are utilized for the treatment of leishmaniasis, but most of the available drugs have side effects, therefore it is necessary to discover safer, cheaper, and more effective methods with new modes of action. In this study, an effort has been made to isolate a bioactive leishmanicidal agent from the methanolic extract of *Pistacia integerrima*. The extracted and isolated flavonoids 3,5,7,4′-tetrahydroxy-flavanone (1) showed an excellent antileishmanial effect. Docking studies on two key antileishmanial targets enzymes; dihydrofolate reductase (DHFR) and pteridine reductase (PTR1) revealed that in-vitro activity may be due to the inhibition of these enzymes.

Data Availability

The data associated with this are available in the main text of this paper.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Hassan A. Hemeg was involved in conceptualization and biological investigation; Abdur Rauf wrote and conducted analysis; and Umer Rashid, Naveed Muhammad, and Yahya S. Al-Awthan performed the molecular docking part; Omar

S. Bahattab and Mohammed A. Al-Duais edited biological screening and statistical analysis; Syed Uzair Ali Shah and Rohit Sharma reviewed and edited this paper. All authors read and approved this paper for publication.

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