Antioxidant and Antiplasmodial Activities of Bergenin and 11-O-Galloylbergenin Isolated from Mallotus philippensis

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Two important biologically active compounds were isolated from Mallotus philippensis. The isolated compounds were characterized using spectroanalytical techniques and found to be bergenin (1) and 11-O-galloylbergenin (2). The in vitro antioxidant and antiplasmodial activities of the isolated compounds were determined. For the antioxidant potential, three standard analytical protocols, namely, DPPH radical scavenging activity (RSA), reducing power assay (RPA), and total antioxidant capacity (TAC) assay, were adopted. The results showed that compound 2 was found to be more potent antioxidant as compared to 1. Fascinatingly, compound 2 displayed better EC50 results as compared to α-tocopherol while being comparable with ascorbic acid. The antiplasmodial assay data showed that both the compound exhibited good activity against chloroquine sensitive strain of Plasmodium falciparum (D10) and IC50 values were found to be less than 8 μM. The in silico molecular docking analyses were also performed for the determination of binding affinity of the isolated compounds using P. falciparum proteins PfLDH and PfP27. The results showed that compound 2 has high docking score and binding affinity to both protein receptors as compared to compound 1. The demonstrated biological potentials declared that compound 2 could be the better natural antioxidant and antiplasmodial candidate.

1. Introduction

Medicinal plants, their extracts, or the isolated purified constituents have been used extensively as remedy for treatment of several diseases. The flora of Pakistan is quite rich with the naturally gifted medicinal plants. However, very little attention was given to explore the medicinal potentials of such worthy materia medica [1–3]. Various ailments such as Parkinson’s, Alzheimer’s, cancer, inflammation, neurodegeneration, aging, injury to blood vessel membranes, heart, and brain, and a number of other diseases may be caused by the free radicals present in the body. Antioxidants are free radical scavengers that may prevent, protect, or reduce the extension of such damage [4, 5]. A number of chemical species including both synthetic and natural products may act as antioxidants. Plants are considered to be the best source of natural antioxidants [1–3, 6]. Similarly, the various antimalarial and antiparasitic drugs such as quinine and artemisinin were also reported from the medicinal plants [7]. Bergenin and 11-O-galloylbergenin are the two natural products. The biological and pharmacological activities of bergenin are well documented in the literature [8–21]. However, very little work has been reported on the biological potentials of 11-O-galloylbergenin [22, 23] which need to be more explored. In the present study, the abovementioned natural products were isolated from Mallotus philippensis and their antioxidant and antiplasmodial activities were investigated. For confirmation of the experimental results, the computational study was also performed using in silico molecular docking.
2. Materials and Methods

2.1. General Experimental Procedures. H-NMR and C-NMR were recorded at 400 MHz for H and at 100 MHz for C using TMS as internal standard with Bruker DPX-400 instrument in deuterated solutions. Mass spectra were recorded on Agilent 5973N instrument using EI mode. IR spectra were determined using a Jasco A-302 spectrophotometer. UV and UV-visible spectra were recorded using U-3000 (Hitachi, Japan) and SP-3000 PLUS (Optima, Japan) spectrophotometers. For TLC and column chromatography, aluminum sheets precoated with silica-gel 60 F254 (20 × 20 cm, 0.2 mm thick; E. Merck, Germany) and silica gel (200–300 mesh), respectively, were used. The commercial solvents were used for extraction purpose and were redistilled. For the antioxidant and antiplasmodial activities, analytical grade reagents and chemicals were used.

2.2. Plant Material. The stem wood of M. philippensis (Euphorbiaceae) was collected from district Bunner located in the north of Pakistan in July 2006 and identified via Voucher Number 1013 (pup) by Professor Dr. Abdur Rashid, Department of Botany, University of Peshawar, Peshawar, Pakistan.

2.3. Extraction and Isolation. Air dried plant material was chopped, grinded, and extracted three times with commercial ethanol for 72h which afforded 4.32% of the crude extract. From the crude extract, n-hexane soluble fraction was then dried under vacuum which was further removed by solvent extraction with water. The aqueous extract. From the crude extract, n-hexane for 72h which afforded 4.32% of the crude extract. From the crude extract, n-hexane soluble fraction was then dried under vacuum which was further removed by solvent extraction with water. The aqueous extract. From the crude extract, n-hexane soluble fraction was then dried under vacuum which was further removed by solvent extraction with water. The aqueous extract. From the crude extract, n-hexane soluble fraction was then dried under vacuum which was further removed by solvent extraction with water. The aqueous extract. From the crude extract, n-hexane soluble fraction was then dried under vacuum which was further removed by solvent extraction with water. The aqueous extract. From the crude extract, n-hexane soluble fraction was then dried under vacuum which was further removed by solvent extraction with water. The aqueous extract. From the crude extract, n-hexane soluble fraction was then dried under vacuum which was further removed by solvent extraction with water. The aqueous extract.

2.4. Antioxidant Potential. The antioxidant potential of the isolated compounds was determined using DPPH radical scavenging activity (RSA) [1–3, 24], reducing power assay (RPA) [24, 25] and total antioxidant capacity (TAC) assay [26].

2.5. Antiplasmodial Activity. For antiplasmodial activity, the isolated compounds were tested against chloroquine sensitive (CQS) strain of Plasmodium falciparum (D10). Continuous in vitro cultures of asexual erythrocyte stages of P. falciparum were maintained using the reported method [27]. For the quantitative assessment of antiplasmodial activity, parasite lactate dehydrogenase assay was adopted [28]. The IC50 values were obtained using a nonlinear dose-response curve fitting analysis via Graph Pad Prism v.4.0 software.

2.6. Molecular Docking. For the in silico molecular docking study, the crystal structures of receptor proteins were downloaded from the protein data bank, code number P1LDH (P. falciparum lactate dehydrogenase) and PFG27 (gametocyte protein) of P. falciparum. The water molecules were removed and 3D protonation of the receptor molecules was carried out. The energies of the retrieved receptors were minimized using the default parameters of MOE energy minimization algorithm (gradient: 0.05, force field: MMFF94X). For the molecular docking of the isolated compounds, default parameters of MOE-dock program were used. To find the correct conformations of the ligands and to obtain minimum energy structure, ligands were allowed to be flexible. At the end of docking, the best conformations of the ligand were analyzed for their binding interactions.

3. Results and Discussion

3.1. Characterization of the Isolated Compounds. The spectral analyses of the two isolated compounds are summarized as follows.

**Compound 1.** White needles; mp = 237°C; UV λmax (log ε) = 279(4.28); IR (KBr) νmax = 3310, 3350, 1712, 1632, 1609, 1510, 1230 cm−1; H-NMR (DMSO-d6, 400 MHz): δ 6.97 (1H, s, H-7), 5.67 (1H, d, H-10b), 4.98 (1H, dd, H-4a), 3.89 (1H, dd, H-4), 3.81 (2H, d, H-11), 3.75 (3H, s, H-12), 3.62 (1H, m, H-2), 3.48 (1H, dd, H-3); C-NMR (DMSO-d6, 100 MHz): δ 60.0 (C-12), 61.1 (C-11), 70.7 (C-3), 72.1 (C-10b), 73.7 (C-4), 79.8 (C-4a), 81.7 (C-2), 109.4 (C-7), 116.0 (C-10a), 118.1 (C-6a), 140.6 (C-9), 148.1 (C-10), 151.0 (C-8), 163.5 (C-6); EIMS m/z (rel. int.): 328 (34), 208 (100), 237 (70), 170 (30).

**Compound 1** was obtained as white crystals. The mass spectral data of compound 1 gave a molecular ion peak at m/z 328 which leads to a molecular formula of C14 H13 O9. The melting point of 237°C is consistent with the published melting point of bergenin, that is, 238°C. The H-NMR spectral analysis showed a signal for one aromatic proton at δ 6.97 (1H, s) and a signal for methoxy protons at δ 3.8 (3H, s). In the C-NMR spectrum, the signals were observed at δ 163.5 and 60.0 for carbonoyl and methoxy groups, respectively. Compound 1 was characterized as bergenin (Figure 1) by comparing its physical and spectral data with previous literature [23, 29].

**Compound 2.** White amorphous powder; mp = 180°C; [α]D15 = +37.6° (EtOH; c 1.2); UV λmax (log ε) = 277(4.22); IR (KBr) νmax = 3310, 1712, 1632, 1609, 1510, 1230 cm−1; H-NMR (90 MHz, Me2C=O-d): δ 7.21(2H, s, gall-H), 7.10 (1H, s, H-7), 3.90 (3H, s). In the 13C-NMR (90 MHz, CD3OD): δ 81.2 (C-2), 72.2 (C-3), 75.7 (C-4), 81.1 (C-4a), 166.5(C-6), 120.0, (C-6a), 112.0 (C-7), 153.1 (C-8), 143.3 (C-9), 150.0 (C-10), 117.7 (C-10a), 74.7 (C-10b), 65.1 (C-11), 61.3 (C-12), 121.8 (C-1′), 111.1(C-2′), 114.7(C-3′), 140.0(C-4′), 169.2 (C-7); EIMS m/z (rel. int.): 480 (32), 328 (34), 208 (100), 237 (70), 170 (30).

**Compound 2** was interpreted and analyzed as C14H2O13 to be monogalloyl ester of bergenin by its H-NMR, 13C-NMR, and EIMS spectral data and also by acid hydrolysis which gave bergenin and gallic acid. The position of the galloyl group was established by 13C-NMR spectrum and 2D techniques. The carbon signals other than that of C-11 in the glucose moiety of bergenin were assigned as given below. The carbon carrying free hydroxyl group (C-3, C-4, and C-11) was unequivocally distinguished from the others (C-2, C-4a, and C-10b) by the deuterium induced differential isotope shift.
**Scheme 1:** Schematic representation of extraction and isolation of compounds 1 and 2 from *Mallotus philippensis* stem wood.

**Figure 1:** Chemical structure of isolated compounds 1 (bergenin) and 2 (11-O-galloylbergenin).
of the aerial parts of various compounds isolated from the methanolic extract acid (Table 2). In a previous study, the antioxidant activity resultsofbetaint hern owat tivitytos tron g evaluated using a DPPH radical scavenging assay and the measurement. Compound 2 was characterized as II-O-galloylbergenin (Figure 1) [23, 30].

3.2. Antioxidant Activity. In DPPH radical scavenging assay, the isolated compounds 1 and 2 showed 6.858 ± 0.329 and 87.26 ± 1.671 % RSA, respectively when compared with the selected standards whose %RSA were in the range from 92.26 ± 0.547 to 98.35 ± 0.871 (Table 1). The demonstrated %RSA of compound 2 clearly indicates that it is the high potency toward DPPH free radical. Similarly, in the RPA, the reducing power capacity of compound 2 was found to be much higher as compared to compound 1 while being comparable with gallic acid and quercetin as depicted in Table 1. The TAC of the isolated compounds and standards was determined as ascorbic acid equivalent as shown in Table 1. As can be seen from the results, again compound 2 displayed better activity as compared to compound 1 and even α-tocopherol. From the above discussion, compound 2 could be declared as the better antioxidant candidate. The antioxidant properties of various plants extract or their purified constituents are well documented in the literature [1–3, 6, 22, 24, 31–34].

Table 2 shows a comparative analysis of EC 50 values of the isolated compounds and standards using DPPH radical scavenging and reducing power assays. The EC 50 values showed more prominent performance of compound 2 as compared to compound 1. For the studied assays data, the EC 50 values for compound 2 showed better results as compared to α-tocopherol while being comparable with the ascorbic acid (Table 2). In a previous study, the antioxidant activity of various compounds isolated from the methanolic extract of the aerial parts of Vitex agnus-castus Linn. plant was evaluated using a DPPH radical scavenging assay and the results obtained were in the range from no activity to strong activity. However, the IC 50 value was not reported [6]. The results obtained in the present study are comparable with the reported data [22].

3.3. Antipla smoidal Activity. Compounds 1 and 2 were also tested for the in vitro antimalarial activity against the CQS D10 strain of P. falciparum and the results obtained are presented in Table 3. As can be seen, both the tested compounds had displayed good activity even at low concentration with IC 50 values of 6.92 ± 0.43 and 7.85 ± 0.61 μM for compounds 1 and 2, respectively, while IC 50 value of 0.031 ± 0.002 μM was recorded for chloroquine (Table 3). The analogous results were also reported previously for mentioned compounds isolated from the roots of Bergenia ligulata.

3.4. Molecular Docking. The binding interaction of the isolated compounds and P. falciparum proteins (PfLDH and Pfg27) was also investigated using in silico molecular docking. The selected proteins are very important because PfLDH has a role in glycolysis for energy production during asexual cycle, while Pfg27 is vital protein for the gametocyte production during sexual phase of the parasite; thus both proteins are potential molecular targets for antimalarial drugs. The results of molecular docking with compound 1 and

### Table 1: Antioxidant activity of the isolated compounds and standards.

| Tested compounds | % radical scavenging activity (RSA) | Reducing power assay (RPA) | Total antioxidant capacity* |
|------------------|-------------------------------------|---------------------------|-----------------------------|
| Bergenin (1)     | 6.858 ± 0.329                       | 0.055 ± 0.002             | 49.159 ± 3.136             |
| II-O-Galloylbergenin (2) | 87.26 ± 1.671                      | 1.315 ± 0.027             | 951.50 ± 109.64            |
| Ascorbic acid   | 97.85 ± 0.623                       | 3.351 ± 0.034             | 2478.36 ± 173.81           |
| Gallic acid     | 98.12 ± 0.931                       | 1.435 ± 0.031             | 2201.05 ± 152.33           |
| Quercetin       | 98.35 ± 0.871                       | 1.772 ± 0.041             | 2030.29 ± 134.51           |
| α-Tocopherol    | 92.26 ± 0.547                       | 22.026 ± 0.074            | 565.17 ± 25.32             |

Each reading is mean (n = 3) ± SD (standard deviation). For RSA and RPA, 100 and 25 μg/mL, respectively, were used. * As ascorbic acid equivalent (μmol/mg).

### Table 2: EC 50 values of the isolated compounds and standards.

| Tested compounds | Radical scavenging assay (EC 50 (μg/mL)) | Reducing power assay (EC 50 (μg/mL)) |
|------------------|-----------------------------------------|-------------------------------------|
| Bergenin (1)     | 99.807 ± 3.120                          | 24.915 ± 1.326                      |
| II-O-Galloylbergenin (2) | 7.276 ± 0.058                          | 5.208 ± 0.095                       |
| Ascorbic acid   | 6.571 ± 0.303                           | 3.551 ± 0.073                       |
| Gallic acid     | 4.732 ± 0.187                           | 1.542 ± 0.062                       |
| Quercetin       | 4.355 ± 0.099                           | 2.073 ± 0.065                       |
| α-Tocopherol    | 33.675 ± 2.019                          | 22.152 ± 1.153                      |

Each reading is mean (n = 3) ± SD (standard deviation). *EC 50: effective concentration at which 50% of DPPH radicals are scavenged and "EC 50": effective concentration at which the absorbance is 0.4.

### Table 3: The in vitro antiplasmodial activity of the isolated compounds and standard.

| Tested compounds | Antiplasmodial activity (IC 50 in μM) |
|------------------|--------------------------------------|
| Bergenin (1)     | 6.92 ± 0.43                          |
| II-O-Galloylbergenin (2) | 7.85 ± 0.61                          |
| Chloroquine      | 0.031 ± 0.002                         |

Each reading is mean (n = 3) ± SD (standard deviation).
PfLDH showed that compound was bound in the binding pocket of the enzyme, making interactions with the residues Lys198, Arg109, Asn108, and Asn197 (basic, side chain donors and backbone donor). Lys98 interacts with oxygen of one hydroxyl group of ring and Arg109 with other hydroxyl group oxygen while oxygen of third hydroxyl group established interaction with the Asn197 whereas the oxygen atom of the ring interacts with Asn108 (Figure S2 in Supplementary Material available online at http://dx.doi.org/10.1155/2016/1051925). Compound 2 was completely docked in cavity of the enzyme PfLDH and established large number of interactions with the residues Arg185, Ser170, Glu256, Lys173, Val166, Gly165, Thr169, Ala253, and Ala249. In the above docking process, the residue Arg185 formed three interactions, that is, one with oxygen of one benzene group of benzene ring, the second with oxygen of carboxylic group of compound 2, and the third (arene-arene interaction) with benzene ring. The residue Ser170 established two interactions (backbone donor and acceptor) with hydrogen of hydroxyl group of two cyclic rings and one with oxygen of hydroxyl group of benzene ring of compound 2. The residue Glu256 showed interaction with the hydrogen atom of benzene hydroxyl group (side chain acceptor) and Lys173 has two interactions (side chain donor) with the two oxygen atoms of two hydroxyl groups of benzene ring. The residue Gly165 formed one interaction with hydrogen of hydroxyl group of benzene ring and residue Thr169 (side chain acceptor) showed one interaction with hydrogen of one hydroxyl group, Val166 (backbone acceptor) formed two interactions with the two hydrogen atoms of one hydroxyl group of one benzene ring and with the other hydroxyl group of another benzene ring. Ala253 and Ala249 (backbone donor) both expressed interactions with oxygen of hydroxyl group and carboxyl group (Figure S3).

The results of molecular docking of compound 1 and PfG27 protein binding showed that 1 was bound into the binding cavity of protein (PfG27) making interactions with the residues Arg31 (basic, side chain donor) and Asp40. Arg31 interacts with oxygen (carboxyl group) to one side of benzene ring while Asp40 was found in polar interaction with H (hydroxyl group) of compound 1 (Figure S4). Similarly, Arg36 residue also showed prominent interaction with oxygen of hydroxyl group as shown in Figure S5. In case of compound 2, Arg31 residue established arene-cation interaction with one of the benzene rings of compound and the residue Glu134 formed three-side interaction, that is, two sides with hydrogen of two hydroxyl groups and one side with one oxygen group of carboxyl group (Figure S6). Further, compound 2 also showed arene-arene interaction the residue His28, while Arg36 and Glu130 formed interaction with hydrogen of hydroxyl groups (Figures S7 and S8). From the MOE-docking studies, it was observed that, for both the proteins, compound 2 has good agreement of docking score and binding affinity to protein receptors as compared to compound 1 as shown in Table 4. The results demonstrated that the isolated compounds are good enough for their potency and effectiveness against P. falciparum.

### 4. Conclusions

The current study deals with the isolation and characterization of two biologically active compounds from *M. philippensis*. The natural products were found to be bergenin (1) and 11-O-galloylbergenin (2). The isolated constituents were evaluated for their antioxidant and antiplasmodial potentials and from the results it was evident that compound 2 was found to be a potent and effective antioxidant as compared to compound 1 and its synthetic derivatives [8, 30]. The isolated compounds also offered good antiplasmodial activity against the tested *P. falciparum* strain which was further confirmed using *in silico* molecular docking. It is therefore concluded that the demonstrated medicinal properties of the isolated compounds could be used as scaffolds for the generation of advanced natural products and may play a vital role in drug development and design.

### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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