SHORT REPORT

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Rec. Nat. Prod. X:X (202X) XX-XX

records of natural products

Computational Study and Biological Evaluation of Isolated Saponins from the Fruits of *Gleditsia aquatica* and *Gleditsia caspica*

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(Received August 07, 2020; Revised October 09, 2020; Accepted October 10, 2020)

Abstract: Phytochemical study of the ethanolic extract of the fruits of *Gleditsia aquatica* and *Gleditsia caspica* resulted in the isolation of the triterpene saponins; aquaticasaponin A (**1**), aquaticasaponin B (**2**), caspicaoside L (**3**) and caspicaoside M (**4**). Compound (**1**) showed good activity against methicillin resistant *Staphylococcus aureus* (MRSA) (IC_{50} =16.3 µg/mL) and *Staphylococcus aureus* (non-MRSA), (IC_{50} =12.2 µg/mL) and it expressed considerable cytotoxic activity against BT-549 (Human Ductal Carcinoma, Breast), KB (Human Epidermal Carcinoma, Oral) and SK-MEL (Human Malignant Melanoma) with IC_{50} values of 8.3, 10.0 and 3.3 µg/mL, respectively. Compounds (**2**) and (**3**) showed potent cytotoxicity against BT-549 with IC_{50} values of 5.3 and 7.3 µg/mL, and against SK-MEL with IC_{50} values of 4.3 and 3.1 µg/mL, respectively. Compound (**4**) showed good cytotoxicity against KB with IC_{50} value of 7.3 µg/mL. In consistent, the study of molecular determinates of cytotoxic activity of these new scaffolds showed close high docking scores to CD81 (Cluster of Differentiation 81) human antigen which could be of great importance for the development of new cytotoxic candidates. The structure identification of isolated metabolites was carried out using 1D and 2D NMR and mass spectra.

Keywords: Triterpenoidal saponins; cytotoxicity; CD81 human antigen; computational study; MRSA.

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The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products Month-Month 2021EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.202.20.08.1764

1. Plant Source

The fruits of *Gleditsia aquatica* March. were collected from Agricultural Museum, Dokki, Giza, Egypt in March 2001 and the fruits of *Gleditsia caspica* Desf. were collected from Al-Orman Garden, Giza, Egypt in April 2014. Both fruits were kindly identified by Badeia Hassan Aly Dewan, Consultant of Egyptian Flora, Agricultural Museum, Dokki, Giza, Egypt, and by Mrs. Terase Labib, Taxonomist of Al-Orman Garden, Giza, Egypt. Voucher specimens [herbarium No. G-01 for *G. aquatica* and herbarium No. G-02 for *G. capsica*] has been deposited in the Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

2. Previous Studies

Genus *Gleditsia* (Fabaceae) are well known due to a wide range of biological activities [1]. The constituents and activities of the major species of *Gleditsia* have been reported; a literature survey indicates, triterpenoidal saponins [2-6]. *Gleditsia aquatica* March. and *Gleditsia caspica* Desf are perennial shrubs or trees distributed throughout Egypt for ornamental purpose. The cytotoxic activity of the triterpenoid saponins isolated from the fruits of genus *Gleditsia* against different cell lines was previously reported [2]. In our previous studies we reported the antimicrobial and cytotoxic activity of aquaticasaponins A and B isolated from *Gleditsia aquatica* [3] and the cytotoxic activity of caspicaosides L and M isolated from *Gleditsia caspica* [4]. Aquaticasaponins A and B showed the highest degree of antimicrobial activity against *Syncephalastrum racemosum* and *Escherichia coli*, respectively and were exhibited good cytotoxic activity against human breast cancer (MCF-7) and human colon cancer (HCT-116) cell lines. Furthermore, caspicaosides L and M showed variable cytotoxic activity against human breast adenocarcinoma (MCF 7), human ovary adenocarcinoma (A2780) and human colon adenocarcinoma (HT 29) cancer cell lines. Caspicaosides L and M were selective against the normal fibroblast MRC 5. In continuation of our effort to search for metabolites against both methicillin resistant *Staphylococcus aureus* (MRSA) and cytotoxic compounds [7, 8]. The aim of this study is to extent the investigation on the antimicrobial and cytotoxic activity of metabolites isolated from *Gleditsia aquatica* and *Gleditsia caspica* and to perform a molecular docking of these metabolites. Isolated metabolites from *Gleditsia aquatica* and *Gleditsia caspica* showed good activity against both methicillin resistant *Staphylococcus aureus* (MRSA) and different human cancer cell lines. In addition, the aglycon component of aquaticasaponin A, echinocystic acid, is reported to bind to CD81 (Cluster of Differentiation 81) human antigen [9] with Kd of 21 nM. Increased expression of CD81 is linked with poor prognosis, increased progression and cellular migration of several cancers including breast and prostate cancers [10, 11]. The effect of CD81 on tumor growth and cancerous cells' migration may be attributed to the function modulation leading to increase in the number of myeloid-derived suppressor cells and T-regulatory cells [12].

3. Present Study

Using combined chromatographic methods, the ethanolic extract of the fruits of *Gleditsia aquatica* and *Gleditsia caspica* resulted in the isolation of the triterpenoidal saponins; aquaticasaponins A (**1**) and B (**2**) from the fruits of *Gleditsia aquatica* and the triterpenoidal saponins caspicaosides L (**3**) and M (**4**) from the fruits of *Gleditsia caspica* (Figure 1). The structures of these compounds were determined using a combination of 1D (^{1}H, ^{13}C, DEPT) and 2D (DQF-COSY, HMQC, HSQC, TOCSY, ^{1}H-^{13}C-HSQC-TOCSY, HMBC, ROESY, NOESY) NMR experiments and HRESIMS analysis. Compound **1** showed good activity against methicillin resistant *Staphylococcus aureus* (MRSA) with IC_{50} value of 16.3 μg/mL. They also exhibit activity against *Staphylococcus aureus* (non-MRSA) with IC_{50} value of 12.2 μg/mL. Compound **1** exhibited high cytotoxic activity against BT-549 (Human Ductal Carcinoma, Breast), KB (Human Epidermal Carcinoma, Oral) and SK-MEL (Human Malignant Melanoma) with IC_{50} values of 8.3, 10.0 and 3.3 μg/mL, respectively. Compounds **2** and **3** showed potent cytotoxicity against BT-549 with IC_{50} values of 5.3 and 7.3

μg/mL, respectively and against SK-MEL with IC_{50} values of 4.3 and 3.1 μg/mL, respectively. Compound **4** revealed good cytotoxicity against KB with IC_{50} value of 7.3 μg/mL (Table 1). The tested compounds **1**-**4** were found to be devoid of cytotoxicity against mammalian cells.

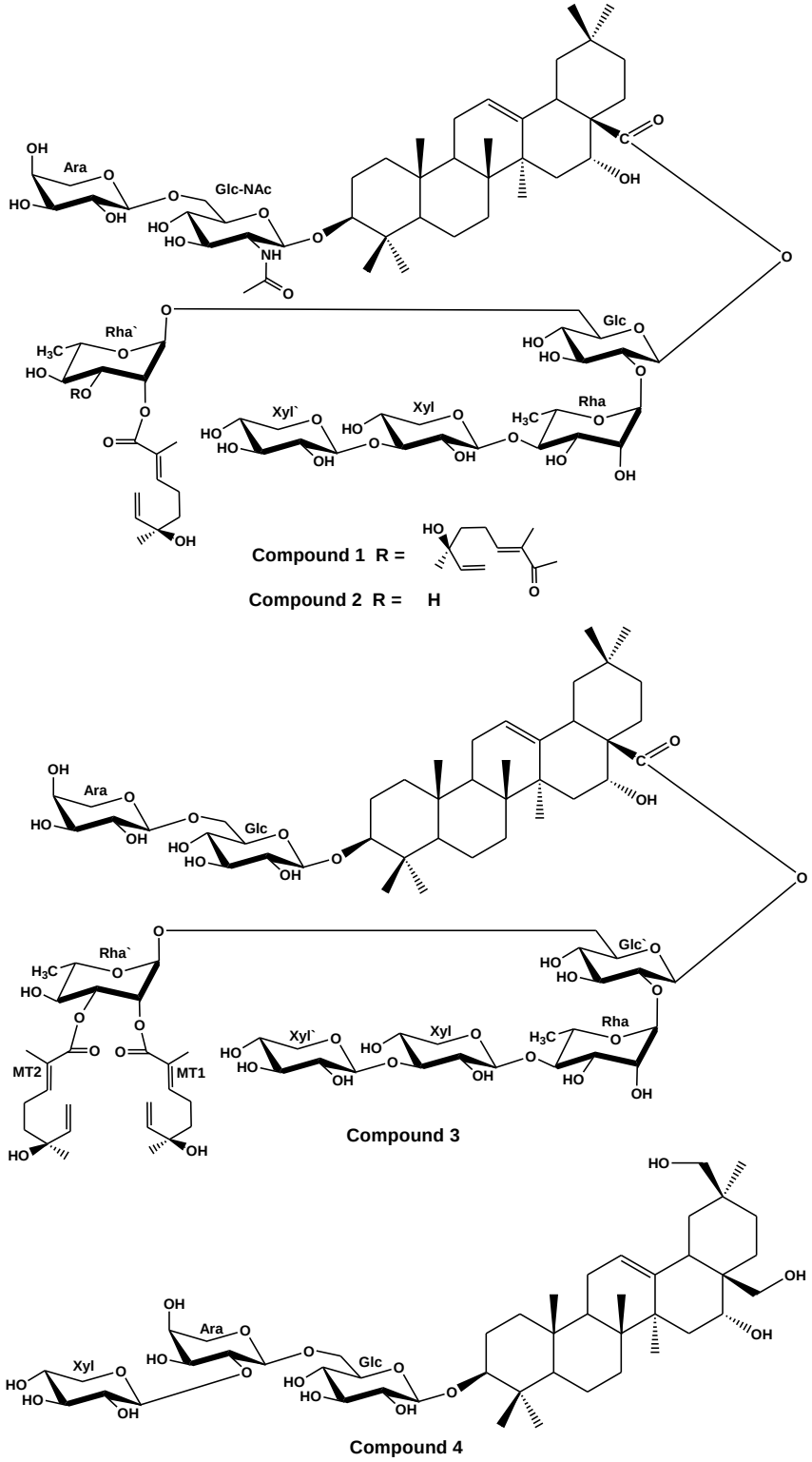

Figure 1. Chemical structures of compounds **1**-4

The cholesterol binding site was considered the ligand pocket in this docking experiment due to the high structural similarity between the aglycon moieties and cholesterol. We did not perform a retrospective docking study. The binding site is wide and may allow for fitting of considerable big molecules. The ligands docked (Figure 2) with high and very similar docking scores of -7.4 kcal/mol and -7.2 kcal/mol. Both ligands and cholesterol showed a strong hydrogen bond with Glu219. The ligands showed hydrophobic and van der Waals contacts with several amino acids in the binding site including Ph17, Asn18, Phe21, Val68, Val71, Met72, Val75 Gly76, Phe94, Leu98, Leu101, Glu105, Val212, Ile215 and Met216. Considering chemical complementarities, the chemistry of the ligands is compatible with that of the receptor, which explains the high binding affinity. The conformational lock of the ligand with reduced number of rotatable bonds, allowed the structures to adopt bioactive-like conformer which is very close to cholesterol in the active site. There are rooms for structural modifications to improve ligand interaction to the target, particularly in place of the sugar portion, however, we must keep the conformational lock and chemical complementarities with the target.

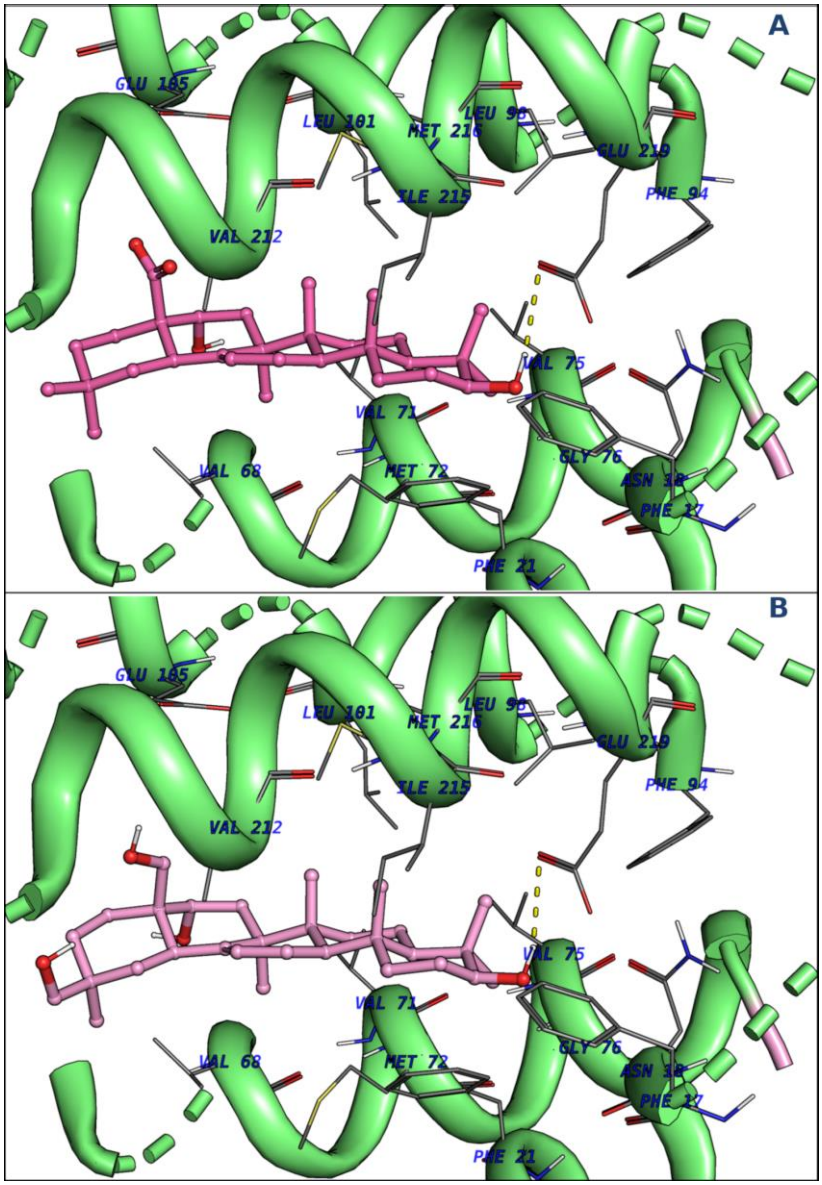

Figure 2. Binding simulations of echinocystic acid (A) and erythrodiol (B) showing the interacting amino acids.

Table 1. Cytotoxic activity of isolated metabolites (Highest test concentration 10 µg/mL)

	IC_{50} in µg/mL					
Compound	**BT-549**	**KB**	**SK-MEL**	**SK-OV-3**	**VERO**	**LLC-PK11**
1	8.3	10	3.3	NA	NA	NA
2	5.3	NA	4.3	NA	NA	NA
3	7.3	NA	3.1	NA	NA	NA
4	NA	7.3	NA	NA	NA	NA
Doxorubicin	1.3	1.5	1.2	1.6	>5	0.5

NA= Not active up to the maximum dose tested 10 µg/mL. cell lines; skin melanoma (SK-MEL), epidermal carcinoma (KB), breast carcinoma (BT-549), ovarian carcinoma (SKOV-3) and two non-cancerous kidney cell lines (LLC-PK11 and Vero).

Acknowledgments

The authors would like to thank the AlMaarefa University for financial support given for this research and thankful for the support from Faculty of Pharmacy, Al-Azhar University.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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