

Antioxidant Activity and In-Silico Study of Anthraquinone Glycosides Extracted from Cassia Fistula Against the Main Protease (7BUY) In SARS-COV2

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With the spreading of Covid-19 and seeking for a drug that helps people around the world to cure this disease. In this article, we used a plant (Cassia fistula) which is rich in anthraquinone glycosides to control the causative agent. Anthraquinone was extracted from Cassia fistula pods using alcohol method. Antioxidant activity of the extracted anthraquinone was analysed by using hydrogen peroxide scavenging assay. The best inhibition assay was 70% at 100mg/ml concentration. The docking study introduced a theoretical explanation for an interaction between two types of anthraquinone glycosides (rhein and aloe-emodin) in Cassia fistula against the main protease (7BZ5) in SARS-COV-2 virus, which gave a good binding energy score as -5.36491489 and -5.48040009 for rhein and aloe-emodin, respectively.

Keywords: Anthraquinone glycoside; Aloe-emodin; Cassia fistula; COVID-19; Rhein.

The widely spread of SARS-COV-2 virus infections around the world, considered as a significant issue, led to a collapse in the health and economic systems. This crisis shows a vital need to discover drugs or innovate vaccines (such as Sinopharm, AstraZeneca and Pfizer) in order to control this disease. Medicinal plants form the main component for many drugs used before to cure several diseases for decades. The term of medicinal plants includes various types of plants used in herbalism. Some plants consider as an important source of nutrition and as a result of that, these

plants recommended for their therapeutic values (Rasool Hassan 2012). *Cassia fistula* Linn. under the Fabaceae family is native to southern Asia but now widely grown in tropical and subtropical areas as an ornament plant due to its beautiful bright yellow flowers. It has some other vernacular names include golden shower, Indian larburnum, and pudding pine tree. *C. fistula*, a national tree of Thailand, is also known as ratchaphruk or Khun in Thai. *C. fistula* pod pulp is widely used in traditional medicines as a purgative/laxative drug and also used against various disorders such

as skin diseases, diabetes, and other ailments (Chewchinda, Wuthi-Udomlert, and Gritsanapan 2013). The pods and leaves of this plant are rich in anthraquinone glycosides, rhein and aloe-emodin are the most important anthraquinone in this plant, these compounds consist of glycon and aglycon part hydrolyzed by β -glucosides of the intestinal flora. Anthraquinone glycosides possess more potent biological activity than the free aglycones (Sakulpanich and Gritsanapan 2009). In addition to this activity, anthraquinone glycosides have another applications in analytical chemistry due to their ability to undergo reversible reactions, conferred by electrophilic character of most of these compounds, and there optical properties, which are directly to their nature and position of substituents and intramolecular and intermolecular interactions, such as hydrogen bonds (Diaz-Muñoz *et al.* 2018). In this research, antioxidant activity and In-silico study of rhein and aloe-emodin (extracted from *Cassia fistula*) against (7BZ5) which is protein SARS-COV2 virus were studied.

MATERIALS AND METHODS

Plant collection

Plant pulps were collected from the local Iraqi market then grind to a fine powder by an electric mill.

Isolation of anthraquinones glycosides

The extraction process was carried on according to (Sakulpanich and Gritsanapan 2008). 10 gm plant powder was macerated with 70% ethanol. The maceration extract was filtered and evaporated to dryness. Then the crude extract was refluxed by using 70% ethanol for 15 min. After cooling the extract, H₂O was added to the mixture and centrifuged for 10 min at 4000 rpm. The supernatant was isolated in a clean flask, then 20 ml of 10.5% FeCl₃·6H₂O was added and refluxed for 20 min, followed by adding 1 ml of HCl and 25 ml ether. Next, the ether layer was isolated and washed with 30 ml H₂O, then adjust to 100 ml with ether and evaporated to dryness.

Hydrogen peroxide scavenging assay

The ability of anthraquinone glycosides extract to scavenge hydrogen peroxide was determined according to the method described by Sasikumar (2014). Briefly, 43 mM hydrogen peroxide solution was prepared in phosphate buffer

(1 M pH 7.4). Different concentrations of sample (100-10 mg/ml) were added to a hydrogen peroxide solution (0.6 ml, 43 mM). The absorbance of hydrogen peroxide at 230 nm was estimated after 10 minutes against a blank solution containing phosphate buffer only (without hydrogen peroxide). Ascorbic acid was used as a standard. Finally, the free radical scavenging was determined by evaluating the % inhibition.

$$\% \text{ inhibition} = \frac{[\text{Control} - \text{Test}]/\text{control}] \times 100}{}$$

Docking study for rhein and aloe-emodin against spike protein (7BZ5) and main protease (7BUY) in COVID-19 virus

In this study, two anthraquinone glycosides abundant in *Cassia fistula* were chosen (Rhein and Aloe-emodin) (Naresh *et al.* 2018). The three-dimensional structures of the main protease (7BUY) in COVID-19 was downloaded from Protein Data Bank (Jin *et al.* 2020). The crystallographic properties illustrated in Table 1. Table 2 shows the chemical structure for rhein and aloe-emodin. The Molecular Operating Environment (MOE software) was used for the docking study. The energy for each protein was minimised, and (Amber 10) used as force field energy. Then, the suitable active site was detected for docking with selected anthraquinone glycosides, which download as SDF format from PubChem. Lipinski's physicochemical parameters rule for each selected anthraquinone glycosides showed in Table 3 (Bouchentouf and Missoum 2020).

RESULTS

Hydrogen peroxide scavenging assay

The scavenging ability of anthraquinone glycosides in hydrogen peroxide is shown in (Fig. 1). The result identified that the highest inhibition percentage was 70.1% belongs to 100mg/ml concentration of anthraquinone, and IC₅₀ of anthraquinone extract from *Cassia fistula* was 61.8% Figure 1.

Molecular docking studies

From five poses by (MOE software) in each docking for rhein and aloe-emodin, one confirmation was chosen according to the best binding energy score and the suitable interaction with the selected protein active site. Table 4 illustrate obtained score for each selected

anthraquinone. While figure 2 illustrate a 2D diagram for interaction between rhein and the main protease (7BUY), which showed one hydrogen interaction possible between carbon-17 in the ligand (Rhein) and receptor 5-ring histidine (41), H-pi interaction with distance 3.81 and energy -1.0 Kcal/mol. In figure 3, a 2D diagram illustrates the interaction between aloe-emodin and 7BUY protein, which showed two hydrogen interactions possible between oxygen-5 in the ligand (aloe-emodin) and receptor amino acid histidin (163), H-acceptor with distance 3.29 and energy -0.6 Kcal/mol. The second hydrogen interaction was

estimated between carbon-17 in the ligand (aloe-emodin) and receptor 5-ring histidine (41), H-pi with distance 3.81 and energy -1.0 Kcal/mol.

DISCUSSION

Cassia fistula is one of the most widespread plants worldwide, especially in India's forests. The whole plant possesses medicinal properties. The experiments illustrate that leaves of the plant have hepatoprotective activity. At the same time, plant pods have antipyretic activity, plant seeds have anticancer activity, while the antioxidant activity

Table 1. Crystallographic properties of S1protein (7BZ5) and Main protease

Protein and enzyme	PDB Code	Classification	Expression system	Resolution	Method	Total structure weight (kDa)	chain
COVID-19 Main protease	7BUY	Viral protein	<i>Escherichia coli</i> B121 (DE3)	1.60 Å	X-ray Diffraction	34.36	A

Table 2. Chemical structures of the two anthraquinones (Rhein and Aloe-emodin)

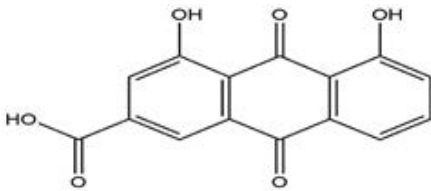
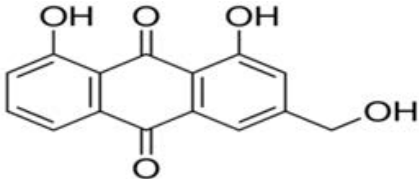
Name of compounds	Chemical structures
Rhein	
Aloe-emodin	

Table 3. Lipinski's physicochemical parameter for Rhein and Aloe-emodin

Compounds	Mol. Weight g/mol	h_logP	h_logS	a_acc	a_don	TPSA (Å ²)	b_rotN	lip_druglike
Rhein	284.2230	2.4404	-3.7005	6	4	111.900	1	1
Aloe-emodin	270.2400	2.4270	-3.4443	5	3	94.8300	1	1

found in leaves, pods and bark (Kumar *et al.* 2017). The results of the antioxidant activity experiment indicate that the anthraquinone extract contains scavenging activity. This activity explains the number of hydroxy groups in the anthraquinone nucleus, which seems to play an essential role in antioxidant activity. Briefly, the effectiveness of anthraquinones with two or three hydroxy groups was higher than those with no hydroxyl groups, according to the structure of rhein and aloe-emodin which contain three hydroxyl group and that may explain the antioxidant activity of them. This activity of these compounds is the secret behind their antiangiogenic and anticancer activities (Mohammed *et al.* 2013; Tripathi *et al.* 2014). For sure, food and plants with antioxidant activity are good choices for human health to prevent the dangerous effect of free radical (Mellado *et al.* 2013). Since December 2019,

SARS-COV-2 virus invasive mostly all the world, finding a vaccine or a drug against this disease an essential issue. One approach to control this crisis is targeting the viral proteins or enzymes to cure this disease. At the same time, there is an apparent lack of laboratory equipment and preventive methods to deal with this virus in many countries worldwide, especially developing countries, in addition to what modern technology offers an intelligent bioinformatics programmes. Thus, this manuscript used a docking technique to illustrate the interaction between the main viral protease and anthraquinone glycosides (Rhein, Aloe-emodin). This enzyme represents one of the most critical targets for the antiviral pharmacological actions against COVID-19. It is essential for the virus due to its proteolytic processing of polyproteins. This enzyme is an attractive target because of its vital role in polyproteins processing, that are translated from the viral RNA (Estrada 2020) (Zhang *et al.* 2020). In silico study showed a good interaction between the two anthraquinones and the main protease (7BUY) with a good score. The research against HIV virus illustrates anthraquinone has a good antiviral activity because these compounds have hydroxyl group, and the anthraquinone with carboxyl group and without hydroxyl group, its loss antiviral activity while the presence of hydroxyl

Table 4. Obtained docking score by anthraquinone glycosides from *Cassia fistula* with 7BUY

Anthraquinone glycosides	Score (Kcal/mol) 7BUY
Rhein	-5.36491489
Aloe-emodin	-5.48040009

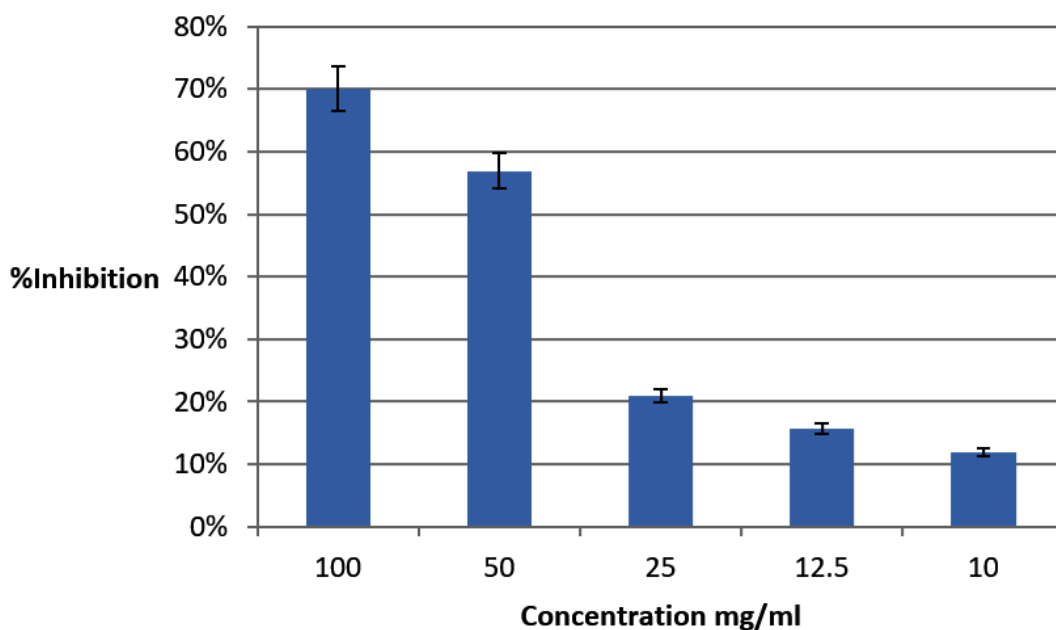


Fig. 1. Hydrogen peroxide radical scavenging activity of Anthraquinone extract from *Cassia fistula*

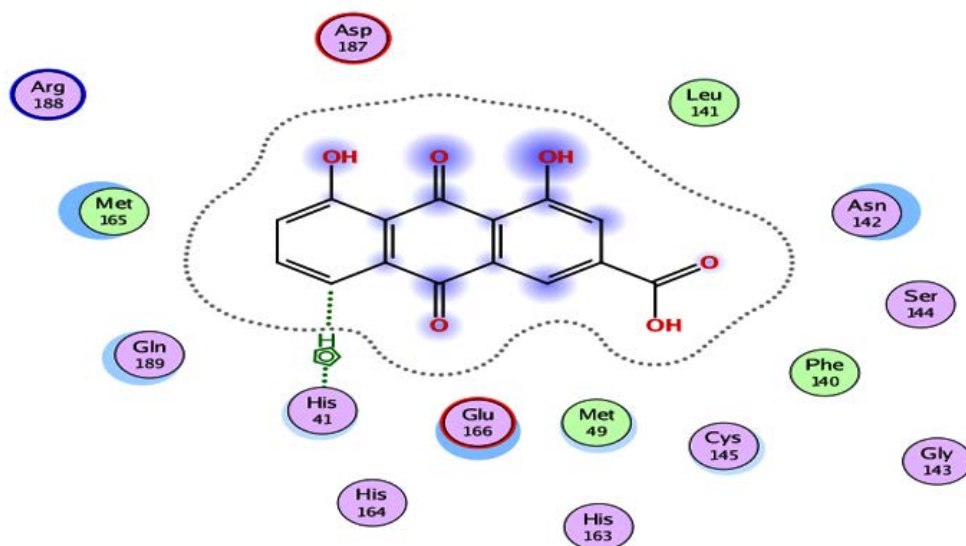


Fig. 2. 2D diagram illustrate theoretical interaction from docking software between Rhein and main protease (7BUY)

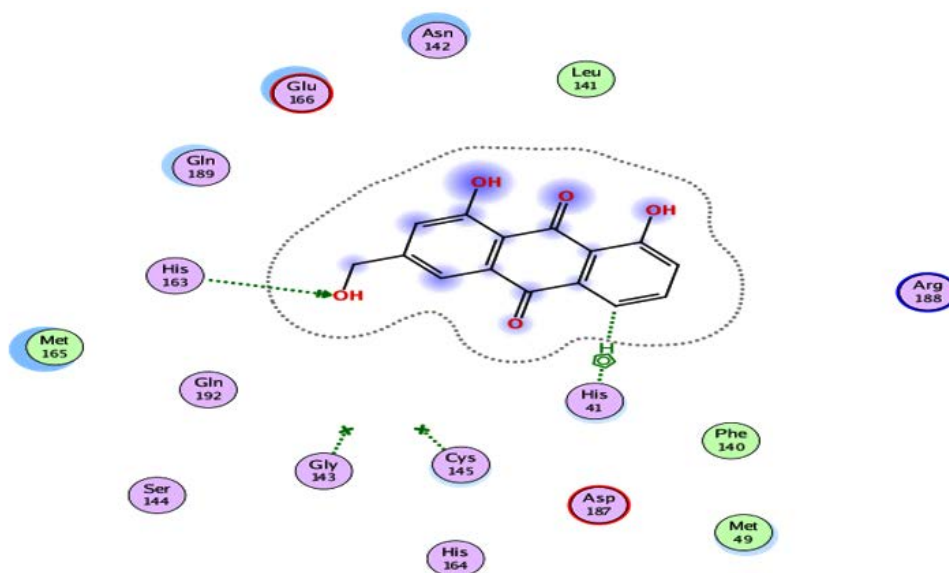


Fig. 3. A 2D diagram illustrate theoretical interaction from docking software between Aloe-emodin and main protease (7BUY)

group give this compounds antiviral activity, and the experiment applied on influenza virus and the aloe-emodin give antiviral activity because its contain thee hydroxyl group and this applied on rhein (Schinazi *et al.* 1990) (Mohammed *et al.* 2013). Interestingly, this article represents the first

step to use these anthraquinone glycosides against SARS-COV-2 virus.

CONCLUSION

Cassia fistula pods is a good source for anthraquinone glycosides which have antioxidant

activity with an inhibition percentage 70% at (100 mg/ml). This article introduce docking study to explain theoretically the effect of two types of anthraquinone in *Cassia fassitula* against the main protease in COVID-19 virus which give a good binding energy score for rhein and aloec-emodin .

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Conflict of interest

None.

REFERENCES

1. Bouchentouf, Salim, and Noureddine Missoum. 2020. “Identification of Compounds from Nigella Sativa as New Potential Inhibitors of 2019 Novel Coronasvirus (Covid-19): Molecular Docking Study</ Strong>.” (April).
2. Chewchinda, Savita, Mansuang Wuthi-Udomlert, and Wandee Gritsanapan. 2013. “HPLC Quantitative Analysis of Rhein and Antidermatophytic Activity of Cassia Fistula Pod Pulp Extracts of Various Storage Conditions.” *BioMed Research International* (2013).
3. Diaz-Muñoz, Gaspar et al. “Anthraquinones: An Overview.” *Studies in Natural Products Chemistry* **58**(July): 313–38 (2018).
4. Estrada, Ernesto. “Topological Analysis of SARS CoV-2 Main Protease.” *Chaos* **30**(6): (2020).
5. Jin, Zhenming et al. “Structural Basis for the Inhibition of SARS-CoV-2 Main Protease by Antineoplastic Drug Carmofur.” *Nature Structural and Molecular Biology* **27**(6): 529–32 (2020). <http://dx.doi.org/10.1038/s41594-020-0440-6>.
6. Kumar, Ajay, Satish, Ibrahim Sayeed, and Karunakara Hedge. “Therapeutic Uses of Cassia Fistula/ : Review.” *International Journal of Pharma And Chemical Research* **3**(1): 38–43 (2017).
7. Mellado, Marco et al. “Antioxidant Activity of Anthraquinones Isolated from Leaves of Muehlenbeckia Hastulata (J.E. SM.) Johnst. (Polygonaceae).” *Journal of the Chilean Chemical Society* **58**(2): 1767–70 (2013).
8. Mohammed, Magdy M. D., Sahar S. El-Souda, Salwa M. El-Hallouty, and Nobuyuki Kobayashi. “Antiviral and Cytotoxic Activities of Anthraquinones Isolated from Cassia Roxburghii Linn. Leaves.” *Herba Polonica*, **59**(4): 33–44 (2013).
9. Naresh, Damuka, Dhammapal Bharne, Paramananda Saikia, and Vaibhav Vindal. “Anthraquinone Rich Cassia Fistula Pod Extract Induces IFIT1, Antiviral Protein.” *Indian Journal of Traditional Knowledge* **17**(3): 474–79 (2018).
10. Rasool Hassan, Bassam Abdul. “Medicinal Plants (Importance and Uses).” *Pharmaceutica Analytica Acta* **03**(10): 2–3 (2012).
11. Sakulpanich, Aurapa, and Wandee Gritsanapan. “Extraction Method for High Content of Materials and Methods/ :” **22**(4): 167–72 (2008).
12. Sakulpanich, Aurapa, and Wandee Gritsanapan. “Determination of Anthraquinone Glycoside Content in Cassia Fistula Leaf Extracts for Alternative Source of Laxative Drug.” *International journal of biomedical and pharmaceutical sciences* **3**(1): 42–45 (2009).
13. Sasikumar, Vadivukkarasi. 2014. “Evaluation of Free Radical Scavenging Activity of Various Leaf Extracts from Kedrostis Foetidissima (Jacq.) Cogn.,” *Biochemistry & Analytical Biochemistry* **03**(02).
14. Schinazi, Raymond F. et al. “Anthraquinones as a New Class of Antiviral Agents against Human Immunodeficiency Virus.” *Antiviral Research* **13**(5): 265–72 (1990).
15. Tripathi, Brijesh et al. “Potential Antioxidant Anthraquinones Isolated from Rheum Emodi Showing Nematicidal Activity against Meloidogyne Incognita.” *Journal of Chemistry*, (2014).
16. Zhang, Linlin et al. “Crystal Structure of SARS-CoV-2 Main Protease Provides a Basis for Design of Improved a-Ketoamide Inhibitors.” *Science*, **368**(6489): 409–12 (2020).