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 the 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 larchnum, and pudding pine tree. C. fistula, a national tree of Thailand, is also known as ratchaphruek 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 α-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 (Díaz-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.

\% inhibition = \[(\text{Control- Test})/\text{control}\] ×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 fasitula 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 formate 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 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
Table 1. Crystallographic properties of S1 protein (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    | *Escherichia coli* Bi21 (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             | ![Rhein](image)     |
| Aloe-emodin       | ![Aloe-emodin](image) |

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 (Å²) | 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

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

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

![Graph showing hydrogen peroxide radical scavenging activity of Anthraquinone extract from *Cassia fistula*.](image)

Fig. 1. Hydrogen peroxide radical scavenging activity of Anthraquinone extract from *Cassia fistula*.
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 fistula against the main protease in COVID-19 virus which give a good binding energy score for rhein and aloe-emodin.

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Conflict of interest

None.

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