In Silico Docking Study of Guttiferones; Q-S from Malabar Tamarind Fruit Rind Against SARS-CoV-2 Omicron Spike Protein with Evaluation of Antioxidant Potential, Phenolic Content and HPTLC Fingerprinting of Its Total Extract

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Research Article

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Abstract

(1) **Background:** The Omicron variant of SARS-CoV-2 has rapidly spread and is now the predominant variant worldwide. Its key feature is its ability to evade immunity from natural infection or vaccines, owing to its numerous mutations in the spike protein. In contrast, medicinal plants have been utilized as alternative therapies to alleviate certain signs and symptoms associated with COVID-19. In this study; Malabar tamarind, which belongs to the Clusiaceae family, was studied for HPTLC fingerprint for its total methanolic extract, quantitative determination of its total phenolics and flavonoids, in-vitro evaluation of its antioxidant potential, followed by molecular docking study of three of its reported natural metabolites; Guttiferones Q, R and S, in order to measure their affinity against the target site of the SARS-CoV-2 Omicron Spike Protein.

(2) **Methods:** Total phenolic content was evaluated by Folin-Catechu assay, flavonoids by aluminum chloride assay. Antioxidant potential was estimated by DPPH assay, while the in silico docking study was processed with the use of Azithromycin as a reference drug.

(3) **Results:** Tamarind exhibited a free radical-scavenging activity of 71.75% inhibition. The molecular docking results suggested that Guttiferone R has the highest binding affinity, alongside predicted binding energy of -8.67 kcal/mol and an RMSD value of 1.07 Å compared to Azithromycin, a reference compound, which has binding affinity of -8.90 kcal/mol and an RMSD value of 1.20 Å.

(4) **Conclusions:** Guttiferone R has the strongest potential as a drug candidate, based on its high binding affinity and low RMSD value, which suggests that it has a stable binding mode.

Introduction

The Omicron variant of SARS-CoV-2 has rapidly spread and is now the predominant variant worldwide. Its key feature is its ability to evade immunity from natural infection or vaccines, owing to its numerous mutations in the spike protein. In contrast, medicinal plants have been utilized as alternative therapies to alleviate certain signs and symptoms associated with COVID-19. The evolution of the SARS-CoV-2 virus and its subsequent global spread have created an unprecedented health crisis. As the virus continues to mutate, new variants have emerged, with the latest being the Omicron variant. Due to the high number of mutations in the Omicron variety, especially in the Spike Protein, concerns have been raised. The Spike Protein is a key component of the SARS-CoV-2 virus that allows it to enter human cells and cause infection. Understanding the structure and function of the Spike Protein is crucial in developing effective treatments and vaccines against COVID-19. Malabar Tamarind (Garcinia cambogia Roxb.), which belongs to the Guttiferae (Clusiaceae) family, is a dicotyledonous tree native to India, Sri Lanka, Africa, and Malaysia. Figure. 1. It is an indigenous tropical plant of India and South East Asia that its fruits have sweet and sour taste and become renowned as weight loss and food supplement. Guttiferones are the most common class of compounds found in plants belonging to the Guttiferae family, which includes genus such as Garcinia, Hypericum, and Clusia. These compounds have a wide range of biological...
activities and have been the subject of much research in recent years. They are polycyclic polyrenylated acylphloroglucinols molecules (PPAPs), abundant in plants of the Malabar tamarind under investigation (3). One of the most well-known guttiferones is garcinol, which is found in the fruit rind of Malabar Tamarind. The fruit's rind contains hydroxyl citric acid (HCA) which is believed to aid in fat-burning and weight loss, and it is also abundant in antioxidants (4). Another important guttiferone is xanthochymol, which is found in the leaves of *Hypericum perforatum*, known as St. John's Wort. According to reports, xanthochymol contains antimicrobial, anti-inflammatory, and antioxidant effects. Additionally, it has been discovered to have anti-cancer agent potential. (5).

**Figure 1. Photograph of Malabar Tamarind**

In this article, three natural metabolites previously reported (6) in Tamarind fruit rind; Guttiferones Q-S, are screened via *in silico* molecular docking against the SARS-CoV-2 Omicron Spike Protein in order to evaluate its biological potential and show the affinity of these compounds against that target site (Fig. 2). In silico approach has become a commonly used computational tool for conducting virtual biological screenings, with this technique, the potential biological activities and estimated affinities of a variety of compounds, including natural products, synthetic molecules, and semisynthetic compounds, can be evaluated. (7). At first, proteins were downloaded from the protein data bank (protein Id: 7T9K). Protein and the natural metabolites were prepared, with minimizing the energy by MMFF94 force field. The molecular docking was done, twenty poses were generated then the best orientations were captured, and affinity scores and RMSD values were collected.

**Figure 2. Structure of Guttiferones Q-S.**

In conclusion, it has been discovered that members of the Guttiferae family of plants contain a diversity of biological activities, such as anti-inflammatory, antioxidant, and anticancer capabilities. More research is required to completely understand their modes of action, safety, and efficacy, despite the fact that they have the potential to be therapeutic agents and have been used in traditional medicine for a range of diseases.

**Materials and methods**

1. **Plant material**

Malabar tamarind fruits rind were purchased in January 2023, from authenticated herbal market, a voucher sample (No. FuPD-4) was kept at the Pharmacognosy Department, Faculty of Pharmacy in Fayoum University.

2. **Preparation of extracts**

In order to produce a homogenized powder; A weighted amount 50 g of the rind of Malabar tamarind had been cleaned and then milled, at room temperature for 6 minutes, using a Moulinex grinder at a speed of
3000 RCF. The resulting powder was subsequently cold macerated with 70% methanol and the mixture was evaporated at 40°C under vacuum pressure, which resulted in the production of 2.65 g of dried crude extract. This extract was used for both phytochemical and biological analyses.

3. HPTLC Fingerprinting

Malabar Tamarind crude methanolic extract sample was weighted, dissolved in methanol with concentration of 6 mg/ml, then filtered. After that, the filtered solutions were applied using nitrogen flow. The operating conditions have been performed as follows; Volume of injection is 2 µL, speed of syringe delivery is 10 s µL⁻¹ (100 nL s⁻¹) and band width is 6 mm with 15 mm distance from the bottom.

CAMAG TLC Scanner 4 (Camag, Muttenz, Switzerland) that is run by WinCATS software, version 1.4.1 (Camag, Muttenz, Switzerland), and that is set to the absorbance mode. A tungsten lamp and deuterium were utilised as the radiation source. We retained the spectrum scan speed at 100 nm/s. The chromatographic plates were scanned at a speed of 20 mm/s with a slit size of 8.00 mm 0.40 mm. Chromatograms underwent densitometric analysis at 280-254-365 nm (1 nm). Silica gel 60 F254 HPTLC plates (20 x 10 cm) were used for the chromatographic separation, which was carried out in a saturated (33% relative humidity) automated development chamber (ADC2, CAMAG).

4. Determination of the total phenolic and flavonoid contents

First, an ultrasonic bath was used to extract a weighed quantity (0.5 g) of each powdered *Garcinia cambogia* Roxb. for 20 minutes. After filtering the extract, methanol was used to bring the filtrate's volume to 50 ml. Aliquots of sample were performed and the assays were done twice.

4.1 Total phenolic content

Folin-Ciocalteu method was utilized for evaluation of the total phenolic content. To create a standard curve, several serial dilutions of gallic acid (20, 40, 60, 80, and 100 mg/L) were used (Fig. 3), 9 ml of distilled water, and 1 ml of each of the tested extract and gallic acid solutions were added to a 25 ml volumetric flask. After 5 minutes, 10 ml of Na₂CO₃ (7%) was added, followed by 1 ml of Folin-Ciocalteu reagent. After carefully mixing the solution, the volume was reduced to 25 ml by the use of distilled water at room temperature, and it was allowed to sit for 90 minutes. A blank experiment was performed using 1 ml of distilled water. UV-VIS spectrophotometer recorded the absorbances at 750 nm. The total phenolic content, in each sample, expressed as mg of gallic acid equivalent (GAE)/100 mg dry weight, was determined from the standard curve (11).

4.2 Total flavonoid content

To quantify total flavonoid content, aluminum chloride colorimetric test was utilized. First, the standard curve was plotted using various concentrations of standard rutin (20, 40, 60, 80, and 100 mg/L), as seen
in (Fig. 4). Then, a 10 ml volumetric flask containing 4 ml of distilled water was filled with 1 ml each of the tested sample and standard solution. After that, 0.3 ml of NaNO2 (5%) was further added, after 5 minutes, the mixture was tested with 0.3 ml of AlCl3 (2%). It was then left for 6 minutes before adding 2 ml of NaOH (1M). The volume was adjusted to 10 ml with distilled water, and the solution was mixed carefully. The absorbance was read at 510 nm against a blank solution prepared using 1 ml of distilled water. The total flavonoid content, expressed as mg of rutin equivalent (RE)/100 mg dry weight, in each of the tested samples was determined from the standard curve (12), control experiment was performed using 1 ml of distilled water.

Gallic acid and rutin standards were used as reference samples, purchased from E. Merk in Darmstadt, Germany. With UV-visible spectrophotometer (Shimadzu UV-1650 PC ); UV spectra were recorded with measuring absorbance in the UV range.

5. Free radicle Scavenging antioxidant activity

The potential of total methanol (70%) extracts of Malabar tamarind fruit rinds to scavenge the free radicals was evaluated by use of 2,2-Diphenyl-1-picrylhydrazyl according to (8). Aliquots of dried crude extract were prepared in a concentration of 1 mg/ml. The reaction was performed in a 96-well plate in triplicate. Each reaction mixture consisted of 10 µl of the sample and 190 µl of DPPH working solution, resulting in 200 µl final volume and a DPPH concentration of approximately 300 µM. Another blank experiment was processed using 10 µl of methanol. After incubation of the samples for about 30 min at 30 ± 2°C, the absorbance was measured at 517 nm wavelength and the percentage of inhibition of oxidation was calculated.

6. Molecular docking study

For docking the tested compounds against SARS-CoV-2 Omicron Spike Protein, Autodock Vina was employed. The binding sites were generated using the co-crystallized ligand within the crystal protein (PDB code: 7T9K) obtained from RCSB. The targeted proteins were prepared by removing water molecules, correcting unfilled valence atoms, adding missing amino acids, and minimizing the protein peptide energy using CHARMM force fields (13). The essential amino acids were selected and made ready for screening. The tested compounds were drawn in 2D using Chem-Bio Draw Ultra17.0 and saved in SDF file format. The tested ligands were protonated, and their energy was minimized using MMFF94 force field with 0.1 RMSD kcal/mol. The minimized structures were then stored for molecular docking. During the refinement, the docking algorithms allowed each molecule to produce twenty different interaction poses with the protein, while the target pocket remained rigid and the ligands remained flexible. The best-fit poses with the histone deacetylase active site were scored for docking (affinity interaction energy). Software named Discovery Studio 2016 visualizer was used to create the 3D orientation. (14)

7. Statistical analysis
One-way ANOVA was used to analyse the biochemical data, which were expressed as means ± SE. Duncan's multiple range tests and least-significant difference test were used to compare the means between groups. The statistical significance was determined by a P-value < 0.05 (Graph Pad Prism 5).

**Results**

1. **HPTLC Fingerprinting**

   It is clear from Figs. 3 and 4 that at least 8 distinct components are found in the developed chromatogram of the methanol extract of Malabar tamarind when it was scanned at different wavelengths. The most prevalent maximum Rf values were detected at 280 nm, determined to be 0.21, 0.31, 0.42, 0.5, 0.52, 0.62, 0.71, and 0.77 with percentage area of 43.64%, 6.23%, 2.29%, 3.74%, 5.94%, 30.8%, 5.48% and 1.88%, respectively. The remaining components are less numerous. As a result, the generated chromatogram will be precise with the chosen Chloroform: Acetone: Formic acid (75:16.5:8.5) solvent system, making it a superior method for the fingerprinting of the tamarind extract. Figures 3 and 4

   Figure 3. **HPTLC Finger print of methanolic extract of Malabar Tamarind at 280 nm.**

   Figure 4. **3Dimensional finger print of methanolic extract of Malabar Tamarind showing different peaks of phytoconstituent**

2. **Determination of the total phenolic and flavonoid contents**

   The quantification of total phenolics in tamarind methanolic extract was carried out using the mg/g gallic acid equivalent while flavonoids by rutin equivalent. Folin-Ciocalteu method evaluated the total phenolics and the total flavonoid contents were estimated by aluminum chloride assay as reported ((8)), Figures, 5 and 6.

   Figure 5. **Standard curve of gallic acid**

   Figure 6. **Standard curve of rutin**

   The total phenolic and flavonoid contents are described as mg of gallic acid and rutin equivalents, (GAE)/100 mg and (RE)/100 mg dry weight, respectively, in each of the tested samples, concentrations are deduced from the standard curves.

   Results revealed that total phenolic contents and flavonoids were 79.45 and 46.99 mg/g) respectively. Results obtained through this quantitative study are collectively represented by the histogram in Fig. 7.

   Figure 7. **Histogram representing the concentrations of phenolic constituents in Malabar tamarind**

3. **Free radical-scavenging activity**
The DPPH photometric assay is a convenient, rapid, and simple method for screening samples for radical scavenging activity. These advantages make it an ideal tool for selecting natural extracts or compounds with potential antioxidant properties for commercial purposes. The mechanism of this assay depends on the ability of antioxidants to pair with the odd electron of the DPPH radical, resulting in bleaching of its absorption. The presence of a hydrogen donating group, such as hydroxyl, is essential, and phenolics are often found in plant extracts due to this structural requirement (9).

The alcoholic extract of *Garcinia cambogia* Roxb. exhibited a free radical-scavenging activity of 71.75% inhibition, which was lower than that of the control rutin (87.9%). This difference may be attributed to the higher phenolic content of *Garcinia cambogia* Roxb. The high phenolic content of the extract may be responsible for this observation, (10). Results are depicted in Fig. 8.

Figure 8. **Histogram representing the free radical scavenging activity of total methanolic (70%) extracts of Malabar tamarind compared to standard rutin**

4. SARS-CoV-2 OMICRON Spike Protein Inhibitor

The reference compound (Azithromycin) formed five Pi-Alkyl interactions with Leu966, Met740, Lys856 and Pro589, additionally interacting with Lys856, Asn978 and Cys743 with three hydrogen bonds by a distance of 2.62, 1.82 and 2.35 Å. Moreover, the cationic NH group interacted with Asp745 with ionic attractive interaction. (Fig. 9).

Figure 9. **3D and surface mapping of Azithromycin (Fig. a, b) against the Omicron Spike Protein.**

Guttiferone-Q predicted eleven Pi-sigma and Pi-alkyl interactions with Val976, Leu966, Lys856, Pro589, Cys590, Pro322, Cys538, and Arg319. Moreover, it’s binding with Asp745 and Thr549 by two hydrogen bonds with bond lengths of 2.68 and 2.06 Å. The total binding energy of the Guttiferone-Q/ Omicron Spike Protein complex exhibited −8.56 kcal/mol versus −8.90 kcal/mol. for the reference compound (Fig. 10).

Figure 10. **3D and surface mapping of Guttiferone-Q (Fig. a, b) against Omicron Spike Protein.**

The binding mode of Guttiferone-R possessed binding energy of -8.67 kcal/mol against Omicron Spike Protein target site. Guttiferone-R formed four Pi-Alkyl interactions with Pro589, Ile587, Leu546 and Leu966, additionally. Guttiferone-R interacted with Asp745, Lys856 and Leu977 by three hydrogen bonds with bond lengths of 2.25, 2.59 and 2.44 Å (Fig. 11).

Figure 11. **3D and surface mapping of Guttiferone-R (Fig. a, b) against Omicron Spike Protein.**

The binding mode of Guttiferone-S possessed binding energy of -8.55 kcal/mol against the Omicron Spike Protein target site. Guttiferone-S bonded with Asp745, Asn978, Leu977 and Arg1000 with four hydrogen bonds with bonds lengths of 1.94, 2.54, 2.56 and 2.37 Å. Additionally, interacted with Leu966 and Val976 by two Pi-alkyl interactions (Fig. 12).

Figure 12. **3D and surface mapping of Guttiferone-S (Fig. a, b) against the Omicron Spike.**

DG, RMSD, interactions of the tested ligands against the targeted sites is summarized in Table 1.
Table 1
(DG, RMSD, interactions) kcal/mol of (tested ligands) against targeted sites.

<table>
<thead>
<tr>
<th>Targets screened</th>
<th>Tested compounds</th>
<th>RMSD value (Å)</th>
<th>Docking (Affinity) score (kcal/mol)</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omicron Spike Protein</td>
<td>Guttiferone-Q</td>
<td>1.37</td>
<td>-8.56</td>
<td>2 11</td>
</tr>
<tr>
<td></td>
<td>Guttiferone-R</td>
<td>1.07</td>
<td>-8.67</td>
<td>3 4</td>
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<tr>
<td></td>
<td>Guttiferone-S</td>
<td>1.21</td>
<td>-8.55</td>
<td>4 2</td>
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<tr>
<td></td>
<td>Azithromycin</td>
<td>1.20</td>
<td>-8.90</td>
<td>3 5</td>
</tr>
</tbody>
</table>

Discussion

The molecular docking results suggest that Guttiferone-R has the highest binding affinity, with a predicted binding energy of -8.67 kcal/mol and an RMSD value of 1.07 Å. Guttiferones S and Q have similar binding affinity, with values of -8.55 and -8.56 kcal/mol respectively but differ in RMSD values (1.21 Å and 1.37 Å respectively). Azithromycin, a reference compound, has a binding affinity of -8.90 kcal/mol with RMSD value of 1.20 Å. These results indicate that Guttiferone-R has the strongest potential as a drug candidate, based on its high binding affinity and low RMSD value, which suggests that it has a stable binding mode. Guttiferones S and Q may also have potential, but their higher RMSD values may indicate a less stable binding mode and the need for further investigation. Azithromycin serves as a reference in the study, with its well-known binding affinity, and can be used to compare and validate the results of the other compounds. Despite Guttiferones potential medicinal properties, more research is needed to fully understand the mechanisms of action of guttiferones and to determine their safety and efficacy in humans.

Declarations

Acknowledgements: Not applicable

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Ethics approval and consent to participate: Malabar tamarind fruits rind were purchased from authenticated herbal market and confirmed in Department of Taxonomy, Faculty of Agriculture, Fayoum University, a voucher sample (No. FuPD-4) was kept at the Pharmacognosy Department, Faculty of Pharmacy in Fayoum University.

Consent for publication: Not applicable.

Competing interests: The authors declare that they have no competing interests.

References


8. Sabry AI. Phytochemical and biological study of certain Conyza species growing in Egypt: PHD. Thesis, Faculty of Pharmacy, Pharmacognosy Department, Beni-Suef …; 2012.


**Figures**

![Figure 1](image)

**Figure 1**

Photograph of Malabar Tamarind
Figure 2

Structure of Guttiferones Q-S.
Figure 3

HPTLC Finger print of methanolic extract of Malabar Tamarind at 280 nm.
Figure 4

3D dimensional finger print of methanolic extract of Malabar Tamarind showing different peaks of phytoconstituent

\[ y = 0.004 \times + 0.130 \]

Absorbance vs. Concentration (mg)

Figure 5

Standard curve of gallic acid
Figure 6

Standard curve of rutin

\[ y = 0.026x + 0.087 \]
Figure 7

Histogram representing the concentrations of phenolic constituents in Malabar tamarind
Figure 8

Histogram representing the free radical scavenging activity of total methanolic (70%) extracts of Malabar tamarind compared to standard rutin.

Figure 9

A

B
3D and surface mapping of Azithromycin (Fig. a, b) against the Omicron Spike Protein.

Figure 10

3D and surface mapping of Guttiferone-Q (Fig. a, b) against Omicron Spike Protein.

Figure 11

3D and surface mapping of Guttiferone-R (Fig. a, b) against Omicron Spike Protein.
Figure 12

3D and surface mapping of Guttiferone-S (Fig. a, b) against the Omicron Spike.

Supplementary Files

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