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Research Article

B-SITOSTEROL: ISOLATION FROM *MUNTINGIA CALABURA* LINN. BARK EXTRACT, STRUCTURAL ELUCIDATION, AND MOLECULAR DOCKING STUDIES AS POTENTIAL INHIBITOR OF SARS-COV-2 M^{PRO} (COVID-19)

RAKESH N CHAUDHARI[1], SHARUK L.KHAN[2]*, RAVINDRA S CHAUDHARY[1], SHIRISH P JAIN[2], FALAK A SIDDUQUI[2]

[1]Department of Pharmacognosy, JES's College of Pharmacy, Nandurbar, Maharashtra, India. [2]Department of Pharmaceutical Chemistry, Rajarshi Shahu College of Pharmacy, Buldana, Maharashtra, India. Email: sharique.4u4@gmail.com

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ABSTRACT

Objective: A novel human coronavirus, labeled as SARS-CoV-2 (COVID-19), causing pneumonia is spreading around the world. At present, there are no specific treatments for COVID-19. β-sitosterol is well known for its multiple biological actions. This research aims to isolate and study the binding affinity of β-sitosterol for SARS-CoV-2 (COVID-19) main protease (M^{pro}).

Methods: Extraction and column chromatography was performed to isolate the β-sitosterol from an n-hexane extract of Muntingia calabura bark followed by thin-layer chromatography (TLC), high-performance TLC (HPTLC), Fourier-transform infrared (FTIR), and ultraviolet–visible spectroscopy. The molecular docking studies were performed on SARS-CoV-2 M^{pro} to determine the binding affinity of the β-sitosterol using PyRx Virtual Screening Tool.

Results: In the present study, preliminary phytochemical screening showed the presence of carbohydrate, steroid, terpenoid, and flavonoid compounds. A total of 115 fractions was collected from column chromatography using benzene as a solvent by an isocratic elution technique. HPTLC fingerprinting analysis showed the presence of β-sitosterol under 366 nm. FTIR characterization was performed of the same fraction which gives the absorption peaks which resembles the β-sitosterol structure.

Conclusion: The overall study concludes this method can be considered as a standard method for isolation of β-sitosterol from *M. calabura* bark. Favipiravir has a less binding affinity, i.e. −5.7 kcal/mol than β-sitosterol which has −6.9 kcal/mol. The number of hydrogen bonds formed by the favipiravir is much more, i.e., 4 than β-sitosterol which formed only 01 hydrogen bonds with SARS-CoV-2 M^{pro}.

Keywords: *Muntingia calabura*, β-sitosterol, SARS-CoV-2 (COVID-19), Molecular docking, High-performance thin-layer chromatography.

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INTRODUCTION

Muntingia calabura, also known as cherry, it has been an essential herb in the Ayurvedic and indigenous medical systems for over 4000 years. Belong to genus Muntingia which contains about 30 species of tropical fruiting trees in the flowering plant family Tiliaceae. *M. calabura* (Muntingiaceae) grows in the tropical and subtropical regions and its parts are used commonly in folk medicine for a varied variety of conditions. According to Ayurveda, varied medicinal properties are attributed to different parts of the mango tree. Cherry is one of the most popular of all tropical fruits. Various parts of the plant are used as a dentifrice, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, laxative, and diuretic and to treat diarrhea, dysentery, anemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, leucorrhoea, hemorrhage, and piles. All parts are used to treat abscesses, broken horn, rabid dog or jackal bite, tumor, snakebite, stings, Datura poisoning, heatstroke, miscarriage, anthrax, blisters, wounds in the mouth, tympanitis, colic, diarrhea, glossitis, indigestion, bacillosis, bloody dysentery, liver disorders, excessive urination, tetanus and asthma, and hermaphrodite [1-3].

COVID-19 is an infectious disease caused by a coronavirus. A new human coronavirus, which has been labeled SARS-CoV-2, began spreading in December 2019 in Wuhan City, China [4]. As of now until 14 April 2020, there were 1,776,867 confirmed cases, 111,828 confirmed deaths, and 213 countries, areas, or territories with cases around the world (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). The World Health Organization declared this disease pandemic. At present, there are no specific vaccines or treatments for COVID-19. However, many ongoing clinical trials are evaluating potential treatments (https://www.who.int/health-topics/coronavirus#tab=tab_1). Favipiravir has recently been approved for a clinical trial to treat COVID-19. Favipiravir is a purine nucleoside which disturbs viral RNA synthesis, was originally developed by Toyama Chemical of Japan [5]. Therefore, in present work, we have taken favipiravir as a reference molecule for the docking study.

METHODS

Plant material

The plant specimen was collected from Gangapur road, Nashik, Maharashtra, India. Dr. A. Benniamin's, (Scientist-C), (Botanical Survey of India, Koregaon Road, Pune), identified and authenticated the voucher specimen of the plant by comparing morphological features. Voucher specimen no. BBJ-1 (Reference number BSI/WRC/Tech./2013).

Preparation of plant material for extraction

Air-dried bark was processed for size reduction using a cutter mill (portable mixer). The crushed material was passed through #40sieves (coarse powder) for uniform particle size, which gave efficient extraction and yield of extract. The 100 g powder was filled in a Soxhlet apparatus and extracted continuously with n-hexane and methanol. The extraction was conceded until the powder becomes colorless. Then, the content of the round bottom flask was kept for the solvent recovery system which promotes the green chemistry extraction methodology. Approximately 100 ml of n-hexane and methanol were recovered by

this process. Then, the extract was subjected for concentration to get the dry extract powder [6].

Phytochemical screening of extract by chemical tests

The phytochemical investigation of the n-hexane extract of *M. calabura* was done using different standard qualitative tests [7]. The results of phytochemical screening of n-hexane extract of *M. calabura* bark for the presence of alkaloids, carbohydrates, steroids, sterol, glycoside, saponins, flavonoids, phenolic compounds, and triterpenoids are presented in Table 1.

Test for alkaloids

Extracted 0.5 g powdered sample with 2.5 ml methanol and 2.5 ml of 2N hydrochloric acid. Then, the filtrate was treated with Meyer's and Wagner's reagents. The sample was recorded negative based on turbidity and color [8].

Test for flavonoids

0.5 g of powdered extract was heated with 5 ml of ethyl acetate, a water bath (40–50°C) for 5 min. The filtrate was then treated with 0.5 ml dilute ammonia solution. A yellow coloration revealed the presence of flavonoids [8].

Test for saponins

1 g of powdered extract boiled in 10 ml of distilled water and filtered the solution. Then, 5 ml of the filtrate was mixed with 5 ml of distilled water and stirred strongly for stable insistent foam. The foaming was mixed with three drops of olive oil and shakes vigorously which forms emulsion, indicated the presence of saponins [9].

Test for glycosides

Keller–Kiliani test – 1 ml of extract was added in 1ml of glacial acetic acid. Then, 1 ml of ferric chloride was added with 1 ml of concentrated sulfuric acid. The green-blue coloration of the solution specified the presence of glycoside [10].

Test for carbohydrates

1 ml of n-hexane extract was added to 1 ml of Molisch's reagent with stirring. 1 ml of concentrated sulfuric acid was added carefully from the side of the test tube. The development of a red or violet color at the inter-phase of the two layers is symbolic of positive test which concludes the presence of carbohydrates [11].

Test for tannins

About 10% alcoholic ferric chloride solution was added in 2 ml of n-hexane extract (1:1). The development of the dark blue color of the solution indicated the presence of tannins [12].

Test for terpenoids

Salkowski test – to 2.5 ml of n-hexane extract, 2 ml of chloroform was added. Then, 2 ml of concentrated sulfuric acid was added slowly to form a layer. A reddish-brown coloration at boundary directed the presence of terpenoids [9].

Test for steroids

To 1 ml of n-hexane extract, add 2 ml acetic anhydride, and 2 ml of concentrated sulfuric acid, color changes from blue to dark green showed the existence of steroids [9].

Evaluation of extractive values

Extractive values are useful for the evaluation of crude drugs and give an idea about the nature of chemical constituents present in them. The amount of extractive a drug yields to a given solvent is often an approximate measure of a certain constituent or group of related constituents the drug contains. Extractive values after the continuous extraction of bark *M. calabura* given in Table 2.

Isolation of different constituents from extract by column chromatography

A cylinder designed glass column comprising the stationary phase (silica gel) is added slowly from the upper end with a liquid solvent (mobile phase) that runs down the column with the help of gravity. This method is used for the decontamination of compounds from a mixture. Once the column is ready, the sample was encumbered inside the top of the column. The mobile solvent is then permitted to flow down through the column. The compounds in mixture have different interactions aptitude with the stationary phase (silica gel), and the mobile phase, in that way, will flow along with the mobile phase at diverse time intervals or degrees. In this way, the separation of compounds from the mixture was achieved. The individual compounds are collected as fractions and analyzed further for structure elucidation [13].

Column chromatography was performed for the isolation of different phytoconstituents by isocratic elution method. The height of the column was 45 cm and the height of silica gel in the column was 30 cm. Benzene was used as a mobile phase. 15–20 ml of each fraction was collected in a test tube and numbered sequentially for additional analysis on thin-layer chromatography (TLC).

TLC

TLC offers partial separation of both organic and inorganic constituents using thin-layered chromatographic plates, particularly useful for testing

Table 1: The details of SARS-CoV-2 main protease (M^{pro}) used (PDB ID-6LU7)

PDB ID	6LU7
Title	The crystal structure of COVID-19 main protease in complex with an inhibitor N3
DOI	10.2210/pdb6LU7/pdb
Authors	Liu, X.; Zhang, B.; Jin, Z.; Yang, H.; Rao, Z.
Deposited on	2020-01-26
Resolution	2.16 Å (reported)
Classification	Viral protein
Organism(s)	Bat SARS-like coronavirus
Expression system	*Escherichia coli* BL21 (DE3)
Method	X-ray diffraction

Table 2: Preliminary qualitative phytochemical screening on extract of *Muntingia calabura* bark

Sr. No.	Phytochemicals	Test name	n-Hexane extract
1.	Alkaloids	Mayer's test	–
		Wagner's test	–
		Hager's test	–
		Dragendorff's test	–
2.	Flavonoids	Lead acetate test	+ + +
		Ferric chloride test	++
		Sodium hydroxide test	+++
		Shinoda test	+++
3.	Saponins	Foam test	–
		Hemolytic test	–
4.	Glycosides	Keller–Kiliani test	+
		Legal's test	+
5.	Carbohydrates	Molisch's test	++
		Fehlings test	++
		Benedict test	++
6.	Tannins	Ferric chloride test	–
		Lead acetate test	–
		Potassium dichromate test	–
		d) Dilute $KMno_4$	–
7.	Terpenoids	Salkowski test	+++
8.	Steroids	Salkowski test	+++
		Liebermann–Burchard reaction	+++

Where; +++: Very positive, ++: Strong positive, +: Fair positive, –: Absent

the purity of portions. Spot of each fraction was applied on activated TLC plates with the help of a capillary tube at a 1/2 inch apart from the bottom of the TLC plate, and the plate was kept in a developing chamber comprising an appropriate solvent system for the precise time until the developing solvent reaches one-fourth of the TLC plate. The plate was taken out from the developing chamber and dried. Compound spots visualized using the iodine chamber for the existence of exact compounds [14,15]. The Rf value of each spot was calculated by the formula: R_f = distance traveled by the sample (cm)/distance traveled by the solvent (cm).

High performance TLC (HPTLC)

The HPTLC method is the fastest separation technique which gives better precision and accuracy.

Sample preparation

N-hexane extract was dissolved in 1ml of chromatographic grade methanol, which was used for sample application on pre-coated silica gel 60F254 aluminum sheets.

Developing solvent system

The satisfactory resolution of peaks was obtained in the solvent system toluene:ethyl acetate:formic acid (7:2:1).

Sample application

The sample application of extract was carried out (8 mm in length and 0.2 µl in concentration for extract) using the spray technique. The sample was applied in duplicate on pre-coated silica gel 60F254 aluminum sheets (5×10 cm) with the help of Linomat 5 applicator attached to the CAMAG HPTLC system.

Development of chromatogram

The chromatogram was established in Twin Trough Glass Chamber 10×10 cm saturated with solvent toluene:ethyl acetate:formic acid (7:2:1) for an extract for 15 min.

Detection of spots

The air-dried plate was viewed in ultraviolet light (Fig. 1). The chromatograms were scanned by a densitometer at 360 nm for extract. The R_f values and fingerprint data were recorded by WIN CATS software.

Fourier-transform infrared (FTIR) spectroscopy

FTIR spectrophotometer is feasibly the commanding device for detecting the types of functional groups existent in the phytochemicals. The wavelength of light absorbed is the significant feature of the functional groups seen in the annotated spectrum. By interpreting the infrared spectrum, the functional groups in a compound can be determined. Dried powder of fractions collected from column chromatography of *M. calabura* bark was used for FTIR analysis. 10 mg of the dried extract powder was compressed in 100mg of KBr pellet, to prepare translucent sample discs [16-18]. The powdered sample of the extract was loaded in FTIR spectrophotometer (Shimadzu, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

Ultraviolet (UV) visible spectroscopy

The color of the compounds directly affects the absorption and the electrons present in molecules of colored solution go through electronic transitions invisible ranges of the spectrum. The n-hexane extract was scanned under UV visible spectral analysis. The extract was centrifuged at 3000 rpm for 10 min and filtered through Whatman filter paper no. 1. The sample was diluted to 1:10 with the same solvent [19,20]. The extract was scanned in the wavelength ranging from 200 to 400 nm using UV spectrophotometer (Shimadzu, Japan) and the lambda max value was detected.

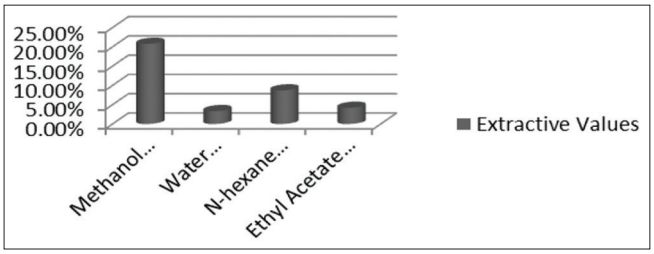

Fig. 1: Graphical presentation of extractive values

Molecular docking

The molecular docking was performed on Lenovo ThinkPad with a 64-bit operating system, Processor: Intel(R) Core(TM) i5-4300M CPU @2.60 GHz 2.59 GHz, RAM: 4GB using PyRx-Virtual Screening Tool.

Ligand preparation

The structures (SDF File) of β-sitosterol and approved drug (Favipiravir) were downloaded from the official website of the U.S. National Library of Medicine PubChem (https://pubchem.ncbi.nlm.nih.gov/). The structure then imported into PyRx 0.8 using open babel tool and energy minimization (optimization) was performed by considering fundamental parameters based on the element, its hybridization, and

Table 3: Analysis of extractive values of *Muntingia calabura* bark

Sr. No.	Evaluation parameter	Value (%)
1.	Methanol-soluble extractive value	20.8
2.	Water-soluble extractive value	3.34
3.	N-hexane soluble extractive value	8.7
4.	Ethyl acetate soluble extractive value	4.20

Table 4: Column chromatography and TLC analysis of *Muntingia calabura* bark extract

Fractions No.	Volume (mL)	Number of spots in TLC	Colour identification of spot on TLC	
			Visual	With iodine chamber
1–5	17	3	Colorless	Brown
6–15	17	2	Colorless	Brown
16–27	15	No spot	-	-
28–31	16	2	Colorless	Orange, yellowish
32–41	18	Multiple	Colorless	Orange, yellowish
42–59	20	No spot	-	-
60–75	20	4	Colorless	Yellowish-brown
76–83	18	2	Colorless	Yellowish-brown
84–115	20	1	Colorless	Yellow

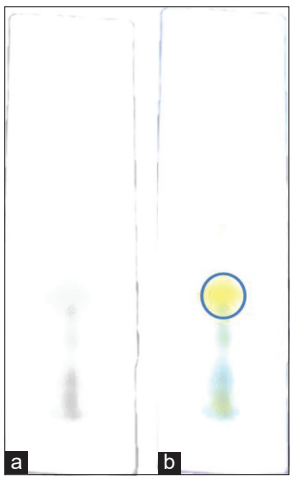

Fig. 2: Thin-layer chromatography of fraction 84–115 (a) daylight, (b) showing single spot after visualizing it in iodine chamber, R_f value=0.53

Table 5: Concern molecular docking details of the molecules

Name of compound	Molecular weight	Binding affinity (kcal/mol)	Ligand energy	No. of hydrogen bonds	Active amino acids
Favipiravir	157.1	−5.7	70.90	4	Gln127, Asp295, Phe08, Asn151, Phe112, Phe294, Thr292, Thr111, Gln110, Ile106
Beta-sitosterol	414.7	−6.9	1658.29	1	Met165, Gln189, Met49, Thr24, Thr26, Thr25, His164, His41, Leu27, Phe140, Leu141, Gly143, Asn142, Ser144, His163, Cys145, His172, Glu166

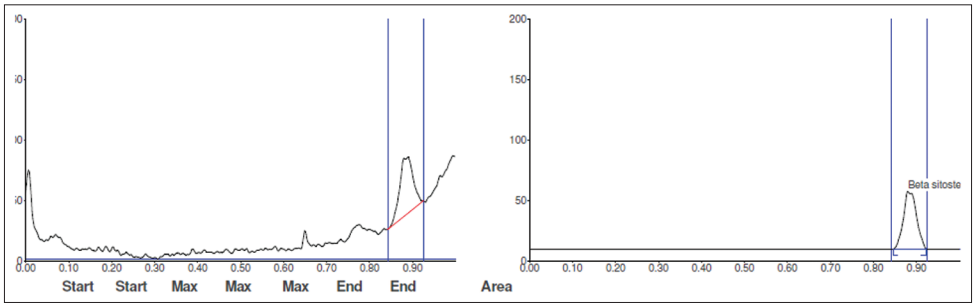

Fig. 3: High-performance thin-layer chromatography fingerprint of fraction 84–115

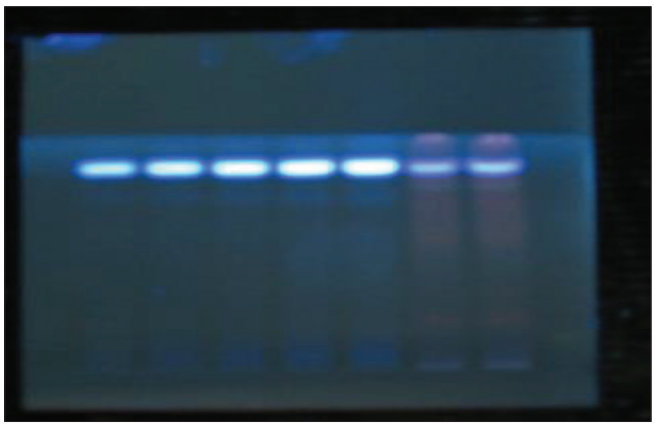

Fig. 4: Isolated substance: β-sitosterol at 366 nm

connectivity, i.e., by Universal Force Field [21]. This ligand was then converted to AutoDock Ligand format (PDBQT).

Target preparation

The AutoDock Vina 1.1.2 in PyRx 0.8 [22] was used to perform the docking studies of β-sitosterol and approved drug (Favipiravir) against the crystal structure of SARS-CoV-2M^{pro}. The recently elucidated crystal structure of SARS-CoV-2M^{pro} was obtained from the RCSB protein data bank (PDB) with PDB ID: 6LU7 (https://www.rcsb.org/structure/6LU7). The viral protein structure was optimized, purified, and prepared for docking with the help of Discovery Studio Visualizer 2019 [23] by removing unwanted water molecules, bound ligands from protein structure, and saved again in PDB file format to the same folder.

The details of SARS-CoV-2 main protease (M^{pro}) used (PDB ID-6LU7) are given in Table 1 obtained from PDB X-ray structure validation report which was released on 02 March 2020.

Docking procedure

The purified SARS-CoV-2 M^{pro} file was loaded to docking software PyRx 0.8 using a load molecule option from the File toolbar. Chain-A was used to perform the docking as it contains the active amino acid residues. The receptor structure then converted to AutoDock macromolecule (PDBQT format) using the right-click option. Binding affinity studies were performed using Vina Wizard Tool in PyRx 0.8. Molecules (PDBQT Files), both ligands (β-sitosterol and favipiravir) as well as the target (SARS-CoV-2 M^{pro}) were selected for docking. For molecular docking simulation, the three-dimensional grid box (size_x=26.3786Ao; size_y=29.0004Ao; size_z=20.8096Ao) was designed using AutoDock tool 1.5.6 with exhaustiveness value of 8. After selecting molecules, the active amino acid residues were selected to define the cavity with the help of the toggle selection spheres option given in PyRx [22]. To occupy all the active binding sites and essential residues, the grid box was aligned properly. The ligands and SARS-CoV-2M^{pro} then subjected for docking to get the finding affinity with each other.

Identification of cavity and active amino acid residues

The active amino acid residues in the protein were identified and noted using BIOVIA Discovery Studio Visualizer (version-19.1.0.18287). All the docking poses, ligand and protein interactions were studied by importing output files into discovery studio which enables us to identify the types of interactions. The chosen cavity is the binding site of natural inhibitor N3, i.e., native ligand in PDB 7LU7.

RESULTS AND DISCUSSION

Phytochemical screening

The therapeutic effects of this plant are due to the occurrence of bioactive chemical components. The preliminary qualitative phytochemical screening of the n-hexane extract of *M. calabura* bark was done to evaluate the presence of phytoconstituents. The presence of various phytoconstituents such as alkaloid, carbohydrate, glycoside, steroid, tannin, terpenoid, flavonoid, and saponins was determined. The extract showed the presence of flavonoids, carbohydrates, terpenoids, and steroids. Traces of glycosides were also found in the n-hexane extract. The phytochemical screening results are shown in Table 2.

Evaluation of extractive value

Extractive values are useful for the evaluation of crude drugs and give an idea about the nature of chemical constituents present in them. Extractive values are mostly beneficial for the determination of exhausted or adulterated drugs. The extractive value defines superiority as well as the pureness of crude drugs [24]. Thus, alcohol and water-soluble extractive values were determined. Methanol extractive value was found to be 20.8%, the water-soluble extractive value was found to be 3.34%. Fig. 1 represents the graphical data of extractive values.

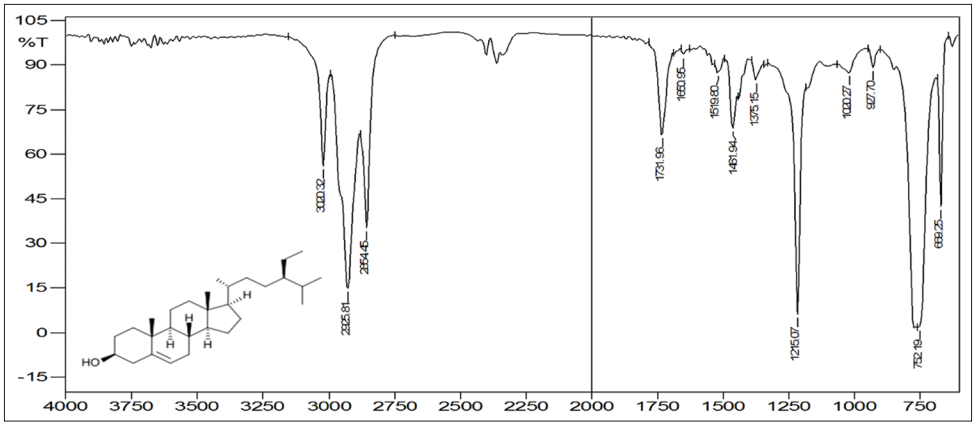

Fig. 5: Fourier-transform infrared spectrum of isolated β-sitosterol

$$\text{Extractive value}(\%) = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100$$

Extractive values after continuous extraction of *M. calabura* bark given in Table 3.

Column chromatography and TLC

A total of 115 fractions were collected from column chromatography according to the colors of bands developed in a column and each fraction was 15–20 ml in quantity. TLC was performed for each fraction to check the number of phytoconstituents present in it. TLC can explain the number of compounds present in a particular solution in the form of spots obtained after visualization. The results are presented in Table 4.

From Table 4, it can be concluded that fraction 84–115 showed a single spot on the TLC plate which indicates the presence of a single chemical component in the fraction, so further structural characterization can be done on the fraction 84–115. TLC was taken using the solvent system in a portion of polar and non-polar proportions. The solvent system used was benzene: methanol:ethyl acetate (5:4:1). After the complete run, TLC plates were visualized by iodine chamber. R_f value was found to be 0.53 (Fig. 2). From the qualitative analysis, it has been concluded that extract has steroids as phytoconstituent. After TLC, the same fraction was forwarded for HPTLC analysis and structural elucidation by UV and IR (Fig. 3).

HPTLC fingerprinting profile

The results from HPTLC fingerprint scanned at wavelength 366 nm for fraction 84–115 of *M. calabura* bark represented in Fig. 4. The HPTLC spectral results have resembled with β-sitosterol. The R_f value was found to be 0.88 at 6 µg which exactly matches the spectral data and peak of standard β-sitosterol. Spectral results are shown in Figs. 2 and 3.

FTIR spectroscopy profile

FTIR spectrum (Fig. 5) showed that the absorption peaks resembles exactly to the functional groups present in β-sitosterol. It showed characteristic bands at 2925.81 cm^{-1} (C-H-stretching, Aliphatic), 3020.32 cm^{-1} (C=C-H-stretching), 1461.94 cm^{-1} (C-H-bending, aliphatic), 1660.96 cm^{-1} (C-C-alkene stretching), 1215.07 cm^{-1} (O-H-bend, Alcoholic), and 1020.27 cm^{-1} (C-O-stretch, alcoholic).

Ultraviolet–visible spectroscopy profile

The qualitative UV-visible profile of fraction 84–115 of *M. calabura* bark, i.e., the isolated β-sitosterol, was taken at the wavelength of 200–400 nm. The lambda max value was observed at 248 nm (Fig. 6).

Molecular docking

The β-sitosterol and favipiravir both successfully docked on SARS-CoV-2 M^{pro} to determine their binding affinity (kcal/mol), i.e., inhibitory potential. Table 5 represents the name of the compounds, molecular

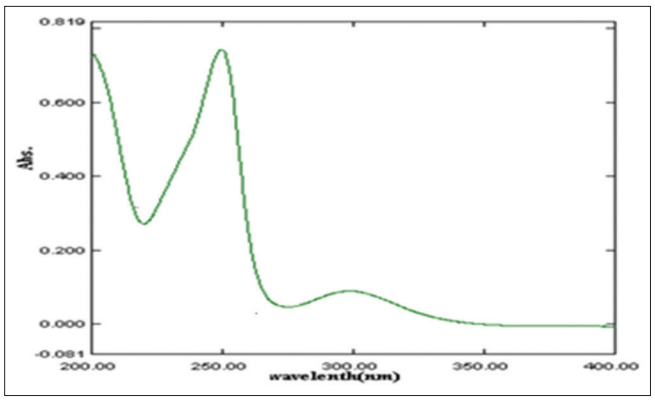

Fig. 6: Ultraviolet spectral maxima of β-sitosterol between 200 and 400 nm

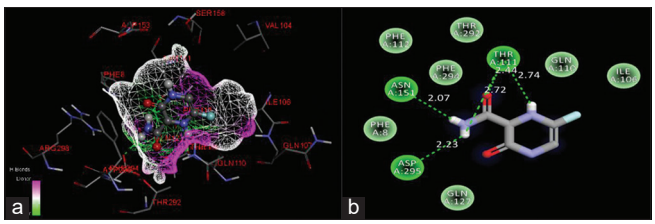

Fig. 7: (a) 3D-docking pose; (b) 2D-docking pose of favipiravir

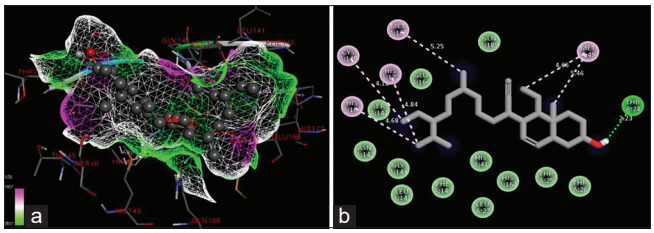

Fig. 8: (a) 3D-docking pose; (b) 2D-docking pose of β-sitosterol

weight, binding affinity (kcal/mol), ligand energy, number of hydrogen bonds formed, and active amino acid residues involved in the interaction.

From molecular docking studies, it has been observed that favipiravir has a less binding affinity, i.e., –5.7 kcal/mol than β-sitosterol which has –6.9 kcal/mol. The number of hydrogen bonds formed by the favipiravir is much more, i.e., 4 than β-sitosterol which formed only 1 hydrogen bonds with SARS-CoV-2 M^{pro}. The binding affinity of β-sitosterol may be more because it interacts with a greater number of amino acids, as shown in Table 5 and forms more van der Waals forces with the target molecule.

Figs. 7a and 8a represent the 3D-docking poses of the molecules favipiravir and β-sitosterol, respectively. Similarly, Figs. 7b and 8b represent the 2D-docking poses of the favipiravir and β-sitosterol, respectively. Favipiravir interacts with amino acids; Gln127, Asp295, Phe08, Asn151, Phe112, Phe294, Thr292, Thr111, Gln110, and Ile106. Whereas, β-sitosterol interacts with Met165, Gln189, Met49, Thr24, Thr26, Thr25, His164, His41, Leu27, Phe140, Leu141, Gly143, Asn142, Ser144, His163, Cys145, His172, and Glu166. Favipiravir forms hydrogen bonds with Asn151 (bond length=2.07A0), Asp295 (bond length=2.23A0), and two hydrogen bonds with Thr111 (bond length=2.72A0, and 2.74A0). Beta-sitosterol forms only one hydrogen bond with Thr24 (bond length=2.23A0). Bond length plays a very important role in the inhibition of the target enzyme or receptor. Although, more the no of hydrogen bonds formed more will be inhibition. While discussing the inhibitory activity, we have considered both binding affinity as well as the number of hydrogen bonds formed.

CONCLUSION

The results of the phytochemical analysis presented that the n-hexane extract of the *M. calabura* bark contains a variety of chemical compounds. The extract showed the presence of flavonoids, carbohydrates, terpenoids, and steroids. Traces of glycosides were also found in the n-hexane extract. The phytochemical analysis of the extract, the identification of liable biological active compounds, and quality standards are essential for the upcoming study.

This present work visualized the successful application of column chromatographic techniques for the isolation of bioactive constituents from medicinal plants. A total of 115 fractions were collected from column chromatography according to the colors of bands developed in a column and each fraction was 15–20 ml in quantity. Fraction 84–115 showed a single spot on the TLC plate which indicates the presence of a single chemical component in the fraction, so further structural characterization can be done on the fraction 84–115. HPTLC studies suggested that isolated compound was β-sitosterol which gave a characteristic peak in HPTLC profile with R_f value 0.88 at 6 µg. Structural elucidation of isolated β-sitosterol was done using FTIR and UV-visible spectroscopy which can be set as a marker for the structural characterization of β-sitosterol, but in the future study, the advanced spectroscopic or chromatographic technique can be used to characterize the β-sitosterol. β-sitosterol is an almost non-polar compound. Therefore, n-hexane can be a better solvent to extract β-sitosterol from the plant by extraction. The overall study concludes that this method can be considered as a standard method for isolation of β-sitosterol from *M. calabura* bark.

From molecular docking studies, it has been concluded that favipiravir has a less binding affinity, i.e. −5.7 kcal/mol than β-sitosterol which has −6.9 kcal/mol. The number of hydrogen bonds formed by the favipiravir is much more, i.e. 4 than β-sitosterol which formed only 1 hydrogen bonds with SARS-CoV-2 M^{pro}. There is an alarming situation created by COVID-19 around the world. The discovery of drugs from a natural source or from repurposing drugs can be great hope in the area of this pandemic. If we develop more optimized models for β-sitosterol, it can create a potential lead to generate inhibitors of SARS-CoV-2 M^{pro} in the future. There is a need to generate more clinical quality data to use it clinically.

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CONTRIBUTION OF AUTHORS'

1. Rakesh N. Chaudhari: Performed all the experimental work related to Pharmacognostic study
2. Sharuk L. Khan: Performed molecular docking studies and manuscript writing
3. Ravindra S. Chaudhary: Provided all the necessary facility required to performed lab work
4. Shirish P. Jain: Final review and validation of the data
5. Falak A. Siddiqui: Compiled the Literature source and data analysis related to Molecular docking.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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