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Research Article

Antioxidant, Anti-Inflammatory, and Anticarcinogenic Efficacy of an Ayurvedic Formulation: Amritotharanam Kashyam

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Amritotharanam Kashyam, a specific Ayurvedic drug, was the focus of the current inquiry to evaluate its efficacy. For liver and digestive-related issues, this medication is suggested. This was obtained from a standard Ayurvedic vendor in Chennai (India), and GC-MS analysis was carried out according to the standard procedure. A few critical biomolecules include benzoic acid, hexadecanoic acid, 6,9-octadecadienoic acid, 9-octadecenoic acid, methyl ester (E)-, heptadecanoic acid, 16-methyl, methyl ester, methyl 18-methylnonadecanoate, tetracosanoic acid, distearin, hexadecanoic acid, and 1-(hydroxymethyl)-1,2-ethanediol ester. The obtained biomolecules exhibited some significant therapeutic functions, including acidification, inhibition of arachidonic acid formation, increase in the aromatic amino acid decarboxylase, suppression of uric acid generation, inhibitors of catechol-O-methyltransferase, urine acidifiers, etc. The anticancer and antiviral potential of these phytocompounds were investigated using molecular docking and dynamics. The phytocompounds pharmacokinetic characteristics were investigated using ADME analysis. Through docking and dynamics simulation, *in silico* tests demonstrated the phytocompounds' inhibitory efficiency against the target proteins. These functions reasonably relate to the medicinal function of Amritotharanam Kashyam. The MTT assay findings demonstrated this medication's anticancer effects. The ability to be an effective drug is demonstrated by its antioxidant, anti-inflammatory, and membrane-stabilizing properties.

1. Introduction

Medicinal plants are currently gaining great attention as possible sources of anticancer medicines and are frequently employed due to the availability of materials, affordability, relatively low cost, and few or no side effects, wide application, and therapeutic efficacy, which has expedited scientific study. The World Health Organization (WHO) encourages the use of traditional medicines because they are safe and effective for these reasons [1]. Amirtotharanam Kashyam is an effective Ayurvedic formulation to treat flu, coughs, cold, and inflammatory diseases and cures various liver disorders. It is effective in recovering liver cells after severe liver diseases such as cirrhosis or hepatitis. This also helps in improving appetite and digestion. This medicine is prepared by adding the powders of *Tinospora cordifolia* (Guduchi), *Zingiber officinale* (Ginger), and *Terminalia chebula* (Haritaki) at the ratio of 2 parts: 6 parts: 4 parts and boiling the mixture with 8 parts of water. The boiling is carried out till the quantity becomes $1/4^{th}$. It is then cooled and preserved in air-tight bottles for medicine. It is prescribed at 12–24 ml with an equal quantity of water and a small spoon of sugar. This medicine references the Ayurvedic treatise, Sahasra Yoga Kasaya Prakarana, Chikitamanjari. The three substances are extensively utilized in home medicines, and substantial research has been carried out on their therapeutic properties.

Ginger (*Zingiber officinale*): ginger is the most frequent home remedy for coughs, colds, and indigestion. Its therapeutic properties have been well-documented [2]. During pregnancy, [3] it has documented its antioxidant effects. Ginger can help with nausea and vomiting [4]. By inhibiting calcium channels, it lowers blood pressure [5]. Guduchi (*Tinospora cordifolia*): *T. Chebula* bark, rind, galls, and other parts of the common Triphalachoornam have been found to have antimicrobial, antioxidant, antidiabetic, anti-inflammatory, hepatoprotective, antiarthritic properties, as well as antiproliferative, antimutagenic, radio and cardioprotective, antispasmodic, antiviral properties, immunomodulatory, and hypolipidemic [6]. Haritaki (*Terminalia chebula*): antidiabetic, antispasmodic, antiperiodic, antiarthritic, antioxidant, anti-inflammatory, antiallergic, antileprotic, antistress, antimalarial, immune-modulatory, antineoplastic, and hepatoprotective effects are some of the therapeutic qualities of this plant [7]. The present work deals with the efficacy determination of Amritotharanam Kashyam using various parameters. The computer-aided drug design (CADD) method helps to identify prospective lead compounds and contribute to the development of potential therapeutics for a wide variety of ailments. A low-cost, time-saving, quick, and automated approach. It provides information about the patterns of small molecules interacting with targeted protein molecules (drug-receptor) interactions [8]. In GC MS analysis to determine the molecules present in medications and to try to comprehend the types of molecules present and their potential medicinal functions that might contribute to Amritotharanam Kashyam's medicinal role. MTT assays to understand the anticancer efficacy of this medicine. The antioxidant, anti-inflammatory, and membrane-stabilizing capacity of Amritotharanam Kashyam studies prove its efficacy as a potent medicine. Inhibitory efficacy of phytocompounds is through Molecular docking and dynamics simulation.

2. Materials and Methods

2.1. HPTLC Profile Chromatography Instrumentation. The CAMAG (Muttenz, Switzerland) HPTLC unit is equipped with Linomat 5 automatic sample applicator with a 100 μL syringe, a TLC sample spotter, a twin trough glass chamber for chromatogram plate development, and a TLC scanner 3 for densitometric evaluation of chromatograms, which is integrated winCATS 4 software (Version 1.4.3, Camag) for interpretation of data.

2.2. Chromatography Conditions. The ethyl acetate extract sample was chromatographed on aluminum-backed HPTLC plates (10 × 10 cm), precoated with silica gel $60F_{254}$ (0.2 mm thickness) (Merck). Before sample application, the plates were activated at 60°C for 5 min after prewashing them with methanol. The ethyl acetate extract sample (2, 4, 8, and 12 μl) was spotted as a band with 8 mm width at different tracks at a distance of 11 mm from the bottom and 15 mm from the side edges of HPTLC aluminum sheets with the help of 100 μl syringe (Hamilton, Bonaduz, Switzerland) attached withCamag semiautomatic Linomat 5 applicator (Camag, Switzerland). The nitrogen stream was passed simultaneously to dry the bands. The plate development occurred in the ascending mode in a twin-trough glass chamber (10 × 10 cm), presaturated with 20 ml of mobile phase for 15 min by sealing the chamber air-tight with parafilm. The chromatogram was run up to 8 cm from the sample application point using n-hexane: ethyl acetate: formic acid: acetic acid in different ratios (7.0 : 3.0 : 0.1 : 0.1) (v/v/v/v), as the mobile phase. The solvent system was selected by trial and error to elute the maximum number of compounds. After the development, the solvent front was marked using a pencil, and then the HPTLC plates were dried in an oven at 60°C for 5 minutes to ensure that all mobile phase was removed. Densitometric scanning was performed immediately using Camag TLC scanner 3 with winCATS software (Version 1.4.8) under 254 nm with 5 mm × 0.45 mm slit dimension for qualitative analysis of ethyl acetate extract 20 mm s^{-1} scanning speed, 100 μm/step data resolution. R_f values of the sample were marked and analyzed using the winCATS software. The images were captured with visible UV light at 366 nm and 254 nm by keeping the plate in the photo-documentation chamber (CAMAG REPROSTAR 3). The peak numbers with their height and area, peak display, and peak densitogram were identified.

2.3. GC MS Profile. Amritotharanam Kashyam was bought from a regular Ayurvedic seller in Chennai and submitted to conventional GC MS analysis. Instrument: Gas chromatography (Agilent: GC: (G3440A) 7890A. MS-MS: 7000 Triple Quad GCMS) was outfitted with a mass spectrometry detector.

2.3.1. Sample Preparation. Samples of 100 microliters and 1 mL of appropriate solvents were used to dissolve the compound. For 10 seconds, the solution was forcefully agitated using a vortex stirrer. Gas-chromatography was used to analyse the clear extract.

2.3.2. GC-MS Protocol. The GC MS Column was constructed of DB5 MS (30 mm 0.25 mm ID 0.25 m, 5 percent phenyl, 95% methyl polysiloxane), Electron impact mode at 70 eV, and Helium (99.999 percent) as a carrier gas at a constant flow rate of 1 ml/min. The auxiliary temperature is 290°C, while the injector temperature is 280°C. The ion source has a temperature of 280°C. For fragments ranging from 45 to 450 Da, the oven temperature was set to climb from 50°C (isothermal for 1.0 min) to 170°C (isothermal for 4.0 min), then 10°C/min to 310°C (isothermal for 10 min). The GC's overall running time is 32.02 minutes. The GC-MS Library is employed (NIST and WILEY).

2.4. Chemicals. The following chemicals were used for MTT Assay: DMEM, Antibiotics (Streptomycin, Penicillin), trypsin-EDTA, PBS, and FBS from Gibco (Invitrogen, USA). MTT reagent and dimethyl sulfoxide (DMSO) are from Sigma Aldrich Chemicals Pvt Ltd, USA.

2.4.1. Sample Preparation. 25 ml of Amritotharanam Kashyam was taken in a Petri dish and kept in a lab water bath at 75–85°C for 3-4 hrs until a solid consistency of extract was obtained. Approximately 1000 mg of the extract was dissolved in 10 ml of water and Dulbecco's Modified Eagle Medium (DMEM) for free radical scavenging and cell viability assay.

2.4.2. Cell Line and Culture. The National Centre For Cell Science, Pune, India (NCCS) supplied the breast cancer cell line (MCF-7). Dulbecco's minimum essential medium added with 10% FBS and 1% antibiotics were used to culture the cells. At 37°C, the cells were maintained in a 5% CO_2 environment. T-75 culture flasks were used to cultivate cells, and the studies were conducted at a confluence of 70% to 80%. Once they had reached confluency, the cells were dissociated by 0.05% trypsin-EDTA enzyme. Cells were seeded at a density of 10^4 cells/cm^2 and treated with various concentrations of Amritotharanam Kashyam. The cells were harvested using trypsin after 24 hours of treatment. Each sample was counted in triplicate under a Nikon (Japan) inverted microscope to assess the total cell numbers.

2.4.3. MTT Assay. This test was conducted to understand the anticancer activity of Amritotharanam Kashyam. To determine the cytotoxicity effect of Amritotharanam Kashyam in the breast cancer cell line, the MTT assay was used. Cell viability was measured by MTT assay, a sensitive, accurate, and reliable colorimetric technique. The assay is based on the ability of the cellular mitochondrial dehydrogenase enzyme in living cells to convert the yellow, water-soluble substrate 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) into a dark blue/purple, water-insoluble formazan product. In 96-well plates, the cells were seeded at a density of 5×10^3 cells/well. The media was changed after 24 hours with 100 μl of medium with Amritotharanam Kashyam at various doses and cultured for another 24 hours. The medium from both the control and Amritotharanam Kashyam treated cells was withdrawn. Each well was given 50 μl of MTT (5 mg/ml), and again plate was kept in a CO_2 incubator for 4 hours at 37°C. The MTT was subsequently removed, and then 50 μl of DMSO was used to dissolve purple-colored formazan crystals. An ELISA reader (Bio-Rad) had been used to quantify the purple-blue formazan mixture at 570 nm. Each sample's optical density was compared with the control, and the percentage of cell viability was calculated and plotted in the graphs.

2.5. Determination of Nuclear Morphological Changes of Cells (DAPI Staining). The cell monolayer was washed with PBS and fixed in 3 percent paraformaldehyde for 10 minutes at room temperature for the nuclear morphological analysis. The frozen cells were permeabilized in PBS containing 0.2 percent Triton X-100 for 10 minutes at room temperature before being treated for 5 minutes with 0.5 g/ml DAPI. The apoptotic nuclei were examined using a fluorescence microscope (intensely stained, shattered nuclei, and condensed chromatin).

2.6. Antioxidant, Anti-Inflammatory Assays, and Membrane Stabilizing Assays

(a) Antioxidant assay - ABTS radical scavenging assay ferric reducing antioxidant potential (FRAP) assay

(b) Anti-inflammatory assay - protein denaturation assay

(c) Membrane stabilisation assay

2.6.1. ABTS (2,2′-Azino-bis (3-Ethylbenzothiazoline-6-sulphonic Acid)) Radical Scavenging Assay. Amrithotharam Kashyam's antioxidant capacity was investigated utilizing the ABTS (2,2′-azino-bis-3-ethyl benzothiazole-6-sulphonic acid) radical cation decolorization test. The radical cations of ABTS (ABTS was created by reacting a 7 mmol/L ABTS solution with 2.45 mmol/L potassium persulphate) ($K_2S_2O_8$) [9]. Before usage, the mixture was kept in the dark at room temperature for 12 hours. Ethanol was used to dilute the ABTS + solution. Different concentrations of test samples (5–320 g/ml) and standard, ascorbic acid (5–320 g/ml) were mixed with the diluted ABTS solution (2.0 ml). The reaction mixture was allowed to stand at room temperature for 6 minutes before measuring absorbance at 734 nm with an ultraviolet-visible spectrophotometer. All determinations were made in triplicate. The radical scavenging activity was denoted as ABTS, and the radical scavenging effect was computed using the following equation:

$$\text{ABTS radical scavenging effect (\%)} = \left[\frac{(\text{A0} - \text{A1})}{\text{A0}}\right] \times 100, \quad (1)$$

where A0 is the absorbance of control; A1 is the absorbance of the test.

2.6.2. Ferric Reducing Potential Antioxidant Assay. The antioxidant ability of the test samples Amritotharanam Kashyam to convert Fe3+ TPTZ complex (colourless complex) to Fe^{2+}tripyridyltriazine (blue coloured complex) generated by electron-donating antioxidants at low pH was calculated spectrophotometrically. It was calculated spectrophotometrically using a modified approach developed by Benzie and Strain [10, 11]. The change in absorbance at 593 nm is used to monitor this process. At 37°C, 300 mM acetate buffer, 10 ml TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ were mixed to make the Ferric reducing antioxidant power (FRAP) reagent. 3.995 ml of newly made working FRAP reagent was pipetted and carefully mixed with varying amounts (5–320 g/ml) of test samples and standard, Ascorbic acid (5–320 g/ml). When the ferric tripyridyl triazine (Fe^{3+} TPTZ) complex was reduced to ferrous (Fe^{2+}) form after 30 minutes at 37°C, a vivid blue colour complex was produced, and the absorbance at 593 nm was measured against a reagent blank (3.995 ml FRAP reagent + 5l distilled water). Every judgment was made in triplicate.

2.6.3. Anti-Inflammatory Assay through Protein Denaturation Assay. Amirthotharam Kashyam's anti-inflammatory potential was determined using a protein denaturation assay. The experiment was carried out with minor modifications by Gnana and colleagues in 2011 [12, 13]. The reference medicine, diclofenac sodium, was dissolved in dimethyl sulfoxide (DMSO), and the sample was diluted using phosphate buffer (0.2 M, PH 7.4). All solutions had a final DMSO concentration of less than 2.5 percent. The Test Solution (4 ml) with varying drug concentrations (50–1600 μg/ml) was mixed with 1 ml of 1 mM albumin solution in phosphate buffer and incubated in an incubator at 37°C for 15 minutes. Denaturation was achieved by immersing the reaction mixture in a 60°C water bath for 15 minutes. After cooling, the turbidity was measured at 660 nm. The turbidity of the reference medication was measured at the same concentration. The percentage of denaturation inhibition was estimated from control in which no medication was applied. The typical medication was diclofenac sodium. The percentage inhibition of denaturation was computed using the formula shown as follows:

$$\% \text{ Inhibition} = \left[\frac{(\text{OD of test} - \text{OD of control})}{\text{OD of control}}\right] \text{x } 100. \quad (2)$$

2.6.4. Membrane Stabilisation Assay. Fresh whole human blood (5 ml) was collected and transferred to centrifuge tubes in a heparinized tube. It was centrifuged at 3000 rpm for 10 minutes before being washed with normal saline of equal volume three times. The blood volume was measured and reconstituted as a 40% v/v suspension with isotonic solution (10 mM sodium phosphate buffer). Amritotharanam Kashyam and regular diclofenac sodium were combined in 0.1 ml of 40% RBC solution at concentrations ranging from 50–1600 μg/ml. The control was 0.1 mL of RBC mixed with an isotonic solution on its own. For 30 minutes, the reaction mixture was incubated in a water bath at 56°C. The tubes were cooled to room temperature after incubation. The reaction mixture was centrifuged for 5 minutes at 2500 rpm, and the supernatant absorbance was measured at 560 nm. To calculate the % membrane stabilizing activity, use the formula as follows:

$$\% \text{ Inhibition of hemolysis} = \left[\frac{(\text{OD of test} - \text{OD of control})}{\text{OD of control}}\right] \text{x } 100. \quad (3)$$

2.7. Statistical Analysis. The results will be presented as mean SEM. One-way ANOVA was used to establish statistical significance, followed by a Dunnett's multiple-comparison test with 95% confidence intervals. P values less than 0.05 were regarded as significant.

2.8. Target and Lead Selection. The resultant phytocompounds from GC-MS analysis possess medicinal properties, therapeutic efficacies, and potential inhibitory actions against various tumours and some deadly diseases. This study focuses on the two deadliest diseases, COVID-19 and breast cancer. The target receptors were examined with the selected phytocompounds to analyse their therapeutic efficacies.

2.9. Protein Preparation and Grid Generation. Three-dimensional structures of the three target receptors of SARS-CoV-2 and two receptors of Breast cancer downloaded from Protein Data Bank (PDB Id: 4YOI, 6YB7, 6M17–SARS-Cov-2 and 1M17, 58XT–Breast cancer). Retrieved structures were imported into the Maestro Schrodinger suite, and the receptors were optimized and minimized using the force field OPLS_2005 [8]. Deletion of water molecules and adding hydrogen were performed before grid generation. The glide module generates the grid box of X, Y, and Z dimensions. After the grid generation, prepared ligands were docked with the target receptors.

2.10. Ligands Preparation. The reported phytocompounds from GC-MS analysis were downloaded from the PubChem database. 2D structures of selected compounds were prepared by the LigPrep module using the force field OPLS_2005 [14]; the resultant prepared ligands were of high quality with accurate bond lengths and angles.

2.11. Molecular Docking. By computing the energy of the ligand binding to the protein, docking is a common molecular modelling approach for inserting ligands into the active site of a receptor molecule. The value of this energy determines the biological activity of molecules; the greater the energy, the more effective the medicine based on the receptor will be assessed. The G-score is calculated in Kcal/mol and incorporates the energies of ligand-protein interactions, hydrophobic interactions, hydrogen bonds, internal energy, and pi-pi stacking interactions. The XP's GLIDE modules visualize the investigation of a specific ligand-protein interaction [15].

2.12. Pharmacophore Hypothesis. The pharmacophore hypothesis generated through the Phase module uses a recently built standard Pharmacophore perception algorithm that reverses the conventional paradigm by detecting ligand alignments and then perceiving hypotheses [16]. The pharmacophore characteristics, such as Acceptor (A), Donor (D), Hydrophobic (H), Negative (N), Positive (P), and Aromatic Rings (R), were determined by three chemical structural patterns and were point, vector, and groups as SMARTS inquiries. Three different geometries, each of which defined the physical attributes of the site, were assigned to these patterns. An aromatic ring's directionality is described by a vector that is normal to the ring's plane in this context. It swiftly develops high-quality hypotheses from a few to hundreds of known active ligands using pharmacophore-based shape alignments.

2.13. MM-GBSA Calculation. The energy of optimum-free receptors, free ligands, and ligand-receptor complexes is calculated using Prime MM-GBSA Molecular Mechanics–Generalized Born Surface Area [17]. It also calculates the ligand strain energy by immersing the ligand in a solution generated automatically by the VSGB 2.0 suit. The energy visualization was shown via the principal energy visualizer.

2.14. ADME Properties. The QikProp module was used to determine absorption, distribution, metabolism, excretion, and toxicity (ADME/T) characteristics for best-docked ligand molecules. This software predicts QP log Po/w, QPlogBB, donor HB, acceptor HB, Caco, rule of 5, total percentage of human oral absorption, etc. Lipinski's rule of five evaluates the drug-likeness of a chemical molecule based on biological ingredients and pharmacological features to predict an orally active therapy [18].

2.15. Molecular Dynamics Simulation. Molecular dynamic simulations can be used to verify docking and understand protein-ligand interactions. It enables the analysis of the system's physical evolution through time and is a valuable tool for interpolating between theory and experimentation. MD performed in GROMACS package with 50 ns by using GROMOS96 43a1 force field [19]. The compound with the lowest binding energy was selected for molecular dynamics (MD) simulation. The PRODRG web server was used to assess the ligand parameters. Cl– or Na+ was added to neutralize the system, and the complex was placed in a cubic box of about 1.0 Å. To minimize the complex, NPT and NVT were performed for 100 ps at 300 k, and MD was performed for 50 ns.

3. Results and Discussion

3.1. HPTLC. In HPTLC observation, 12 peaks were observed and two of them indicated the presence of two primary compounds in the sample. Further analysis is required to identify those two major components. The peak at Rf 0.52 indicated 16.92% of compound A, Peak at Rf-0.71 indicated 52.60% of compound B. The results were illustrated in Figure 1.

3.2. GC MS Results of Amritotharanam Kashyam. Figure 2 depicts the GC MS profile of Amritotharanam Kashyam that displayed the retention values, categories of likely compounds, molecular formulas, molecular weight, peak area, and medicinal advantages of each chemical as demonstrated in Amritotharanam Kashyam's GC MS profile. By comparing retention duration and fragmentation pattern with mass spectra in the NIST spectrum library recorded in the GC-MS computer program, metabolites were identified (version 1.10 beta, Shimadzu). Figure 2 showed the pharmacological functions of each biomolecule based on Dr. Duke's phytochemical and ethnobotanical data source (National Agriculture Library, USA) and others. Benzoic acid, methyl ester, hexadecanoic acid, 6, 9-octadecadienoic acid, 9-octadecenoic acid, methyl ester, (E)-, heptadecanoic acid, 16-methyl-, methyl ester, methyl 18-methylnonadecanoate, tetracosanoic acid. These compounds have far-reaching biological functions, which bodes well for Amritotharanam Kashyam's position as a powerful treatment for which it is given.

3.3. MTT Assay. The MTT assay revealed that Amritotharanam Kashyam had good cytotoxic action against the development of nonsmall cell lung cancer cells in A549 cell line. Amritotharanam Kashyam-treated cells exhibit decreased cell proliferation in MTT assays. Figures 3 and 4 clearly showed that as the concentration of Amritotharanam Kashayam increased, the cell viability reduced almost uniformly. This clearly indicated the cytotoxicity of Amritotharanam Kashayam on cancer cells.

3.4. Nuclear Morphological Changes of Cells (DAPI Staining) Assay. The microscopical observation displayed a decline in the count of the cells and morphological alterations (Figure 4). In this study, we used two different doses to treat the cell line. The increase in the dose concentration declines the cell quantity compared to control cells. Fluorescence microscopic studies indicate abnormal cell boundaries, irregular nucleus shape and enlargement of the cell nucleus. These cell morphological changes and nucleus mutilation have been recovered in the drug-treated cells compared to control cells.

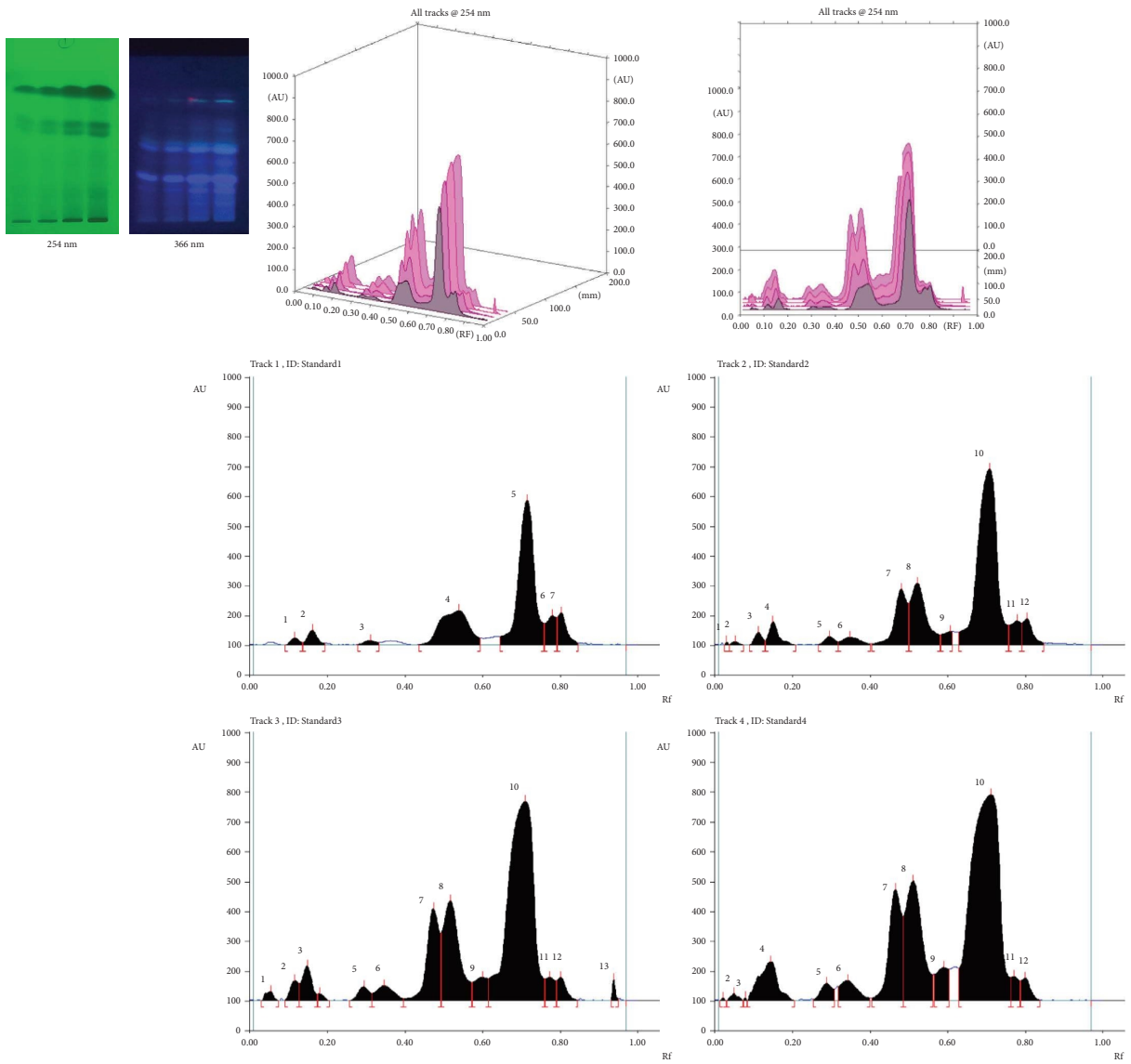

Figure 1: HPTLC-Profile data result.

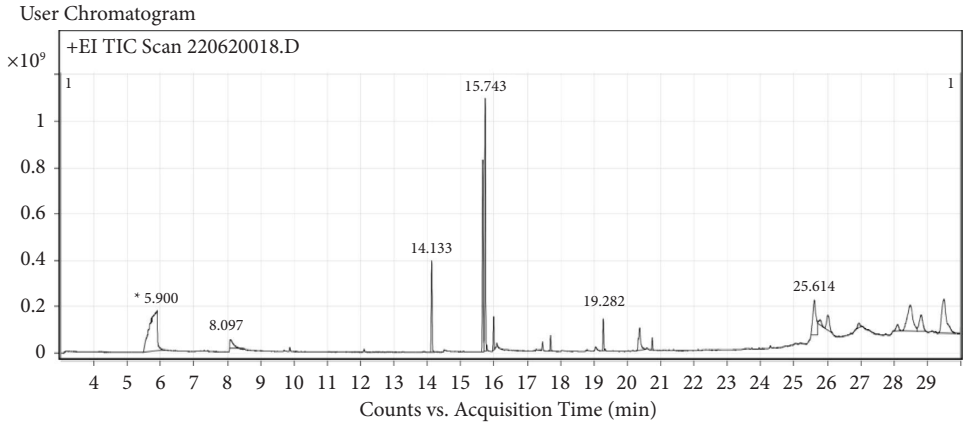

Figure 2: Indicates the GC MS profile of Amritothararam Kashyam.

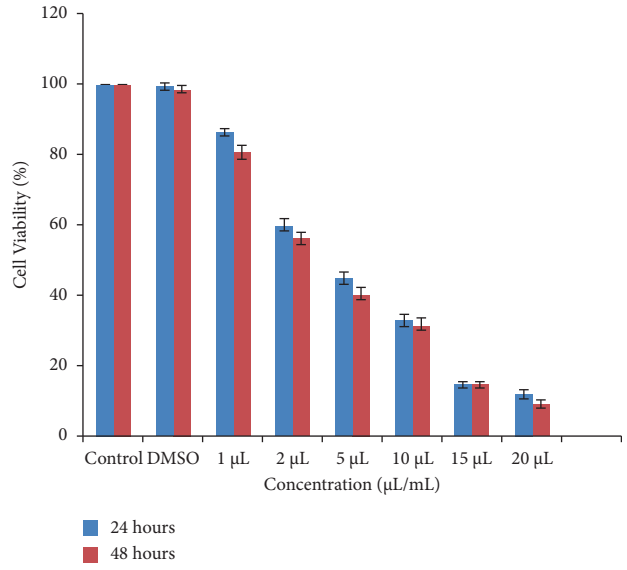

Figure 3: MTT assay comparative chart for 24 hr and 48 hr for Amritotharanam Kashyam.

3.5. Antioxidant, Anti-Inflammatory, and Membrane Stabilizing Potential of Amritotharanam Kashyam. The standard drug ascorbic acid was used as a standard for ABTS and FRAP antioxidant assay. In the case of anti-inflammatory assays such as protein denaturation assay and membrane stabilisation assay; diclofenac sodium is used as standard. It was conducted to have a better comparative study with the test sample, Amritotharanam Kashyam. The sample Amritotharanam Kashyam and standard concentrations are 5, 10, 20, 40, 80,160, and 320 μg/ml for all antioxidant assays, where 50, 100, 200, 400, 800, and 1600 μg/ml concentration was taken for anti-inflammatory assay. The average value of the reactions performed in triplicate was obtained and plotted against the different concentrations of Amritotharanam Kashyam and its standards. The IC_{50} value that is half-maximal inhibitory concentration was calculated from the R^2 equation obtained from a linear thread line from the respective graph of concentration of Amritotharanam Kashyam/standard against % inhibition and activity values. Figure 5 clearly showed that the IC_{50} value for the ABTS radical scavenging effect of Amritotharanam Kashyam was 58.27 μg/ml, and ascorbic acid was 33.43 μg/ml. Amritotharanam Kashyam showed more than four times less radical scavenging effect than standard.

The FRAP assay result was presented in Figure 6. An increase in value was noted in an increase in concentrations of both test and standard. The results indicate that Amritotharanam Kashyam showed comparatively more FRAP activity than standard ascorbic acid. The IC_{50} value for protein denaturation activity of Amritotharanam Kashyam and standard, diclofenac sodium was 160.7 μg/ml and 221.23 μg/ml, respectively, which was obtained from Figure 7. Here the Amritotharanam Kashyam shows better protein denaturation activity than standard. The IC_{50} value for membrane stabilisation activity was obtained from Figure 8, where 525.87 μg/ml and 378.83 μg/ml are IC_{50} Amritotharanam Kashyam and diclofenac sodium, respectively. In membrane stabilisation activity standard is prone to show more activity than a sample.

3.6. Glide Docking. XP Docking was performed using the Glide module, protein-ligand was docked using the XP module, and the conformers were evaluated by *G* score. Docking results declared that the phytocompound 1, 2, 3-benzenetriol exhibited the highest docking score among other phytocompound when examined with the SARS-CoV-2 and breast cancer target proteins. Covid proteins such as 4YOI, 6YB7, and 6M17 showed docking scores of about −7.325, −5.621, and −6.814 Kcal/Mol; furthermore, targets of breast cancer 1M17 and 5DXT showed the docking score of about −6.840 and −6.932 Kcal/Mol respectively. The interacting residues interacted with 1, 2, 3-benzenetriol was GLN_167, GLU_166, LEU_52, TYR_128, GLU_738, THR_766, GLU_849, and VAL_851 respectively. Interacting residue between lead compound 1, 2, 3-benzenetriol and 4YOI is GLN amino acid at the position of 167. Interacting residue between is 1, 2, 3-benzenetriol and 6M17 leucine amino acid at the position of 52 and tyrosine amino acid at the position of 128. Interacting residue between is 1, 2, 3-benzenetriol and 6YB7 glutamic acid amino acid at the position of 166. Interacting residue between is 1, 2, 3-benzenetriol and 1M17 glutamic acid amino acid at the position of 738 and threonine amino acid at the position of 766. Interaction profile between 1, 2, 3-benzenetriol and targeted receptor 5DXT shows the interacting residue VAL 851 and GLU 849. The results were illustrated in Figure 9.

3.7. Binding-Free Energy. The binding affinity of ligands to the receptor was estimated using postdocking binding free energy. The results of the prime MM-GBSA analysis revealed that 1, 2, 3-Benzenetriol had ΔGbind of 35.231 (4YOI), 33.214 (6YB7), 34.127 (6M17), 34.563 (1M17), and 32.501 Kcal/Mol, respectively (Figure 9). According to the binding-free energy, the substances may firmly engage in the active site residues of the target proteins to block enzymatically, stop COVID-19 infection, and inhibit breast cancer activity.

3.8. ADME Properties. The ADME properties and pharmacokinetics of phytocompounds play a significant role in their efficacy. Figure 10 reveals the ADME properties of phytocompounds. The Lipinski principles of five were followed for the phytocompounds, which included 500 molecular weight, 10 hydrogen bond acceptors, 5 hydrogen bond donors, and logP values of 5. Also acceptable were physicochemical parameters such as human oral absorption, partition coefficient (QPlogPo/w), and (QPlogBB) values. As a result, the phytocompounds reported values could be deemed powerfully inhibits the COVID-19 infection and Breast Cancer.

3.9. Pharmacophore Hypothesis. Pharmacophore generated through Phase module and the features of lead compound 1, 2, 3-benzenetriol was illustrated in Figure 10.

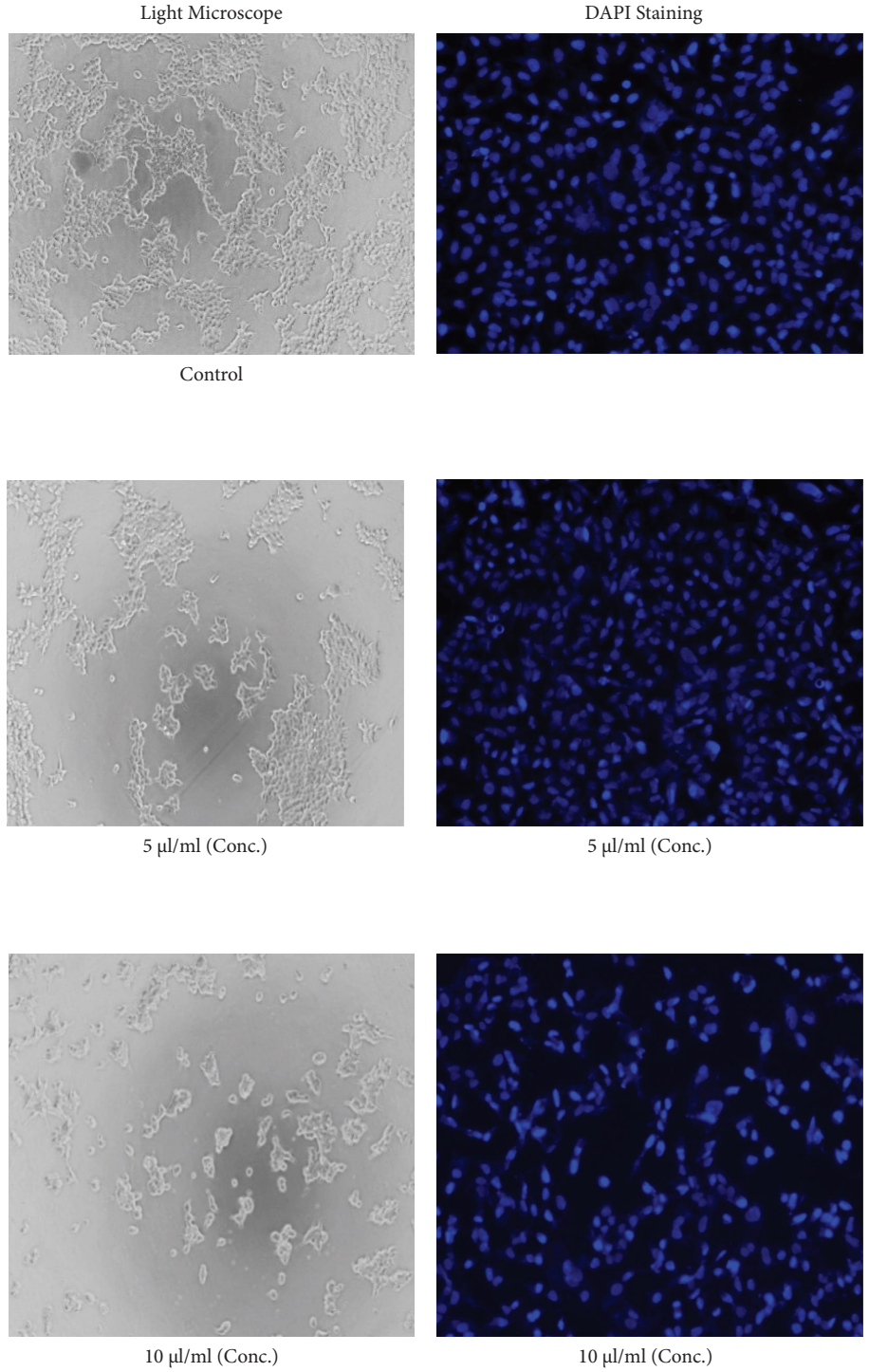

Figure 4: Cell morphology details of the MTT assay for Amritotharanam Kashyam Cell morphology—24 h.

Pharmacophore hypothesis reveals the acceptor, donor and aromatic ring present in the compound. In this lead compound three acceptors, three donors and one aromatic ring present in the active compound.

3.10. MD Simulation. Targeted protein-ligand complex were examined for stability using molecular dynamics simulations from the Gromacs package. According to the docking score, the interaction residues, and the binding affinity between the desired proteins and the lead compound, molecular dynamics simulations were run for 50 ns. The behaviour of macromolecules is typically predicted using MD simulation, which utilizes Newton's equation of motion and classical mechanics to determine the speed and site of each atom in the system under study. The least binding score complex was subjected to MD simulation. RMSD (root mean square deviation), RMSF (root mean square

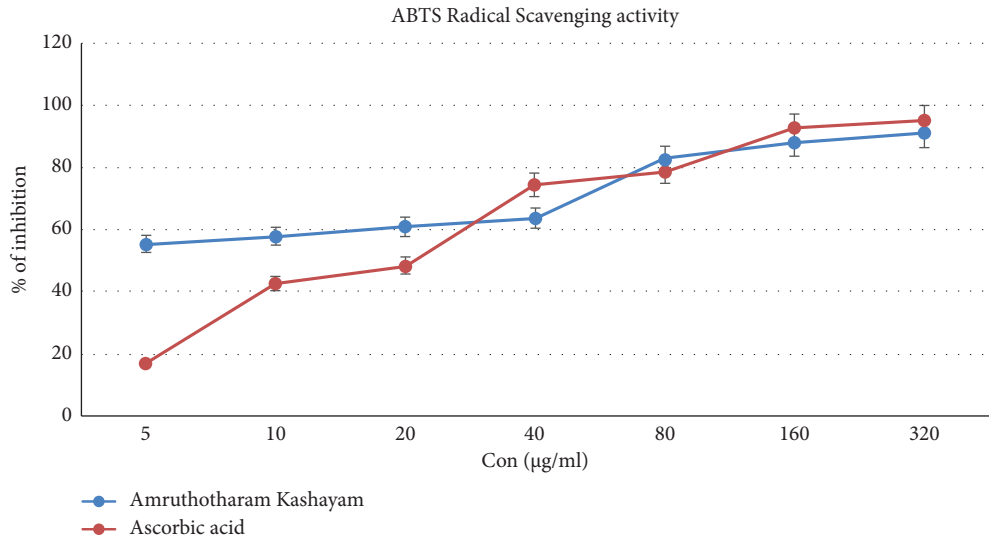

Figure 5: Comparative graphical representation of ABTS radical scavenging activity of Amritotharanam Kashyam and ascorbic acid.

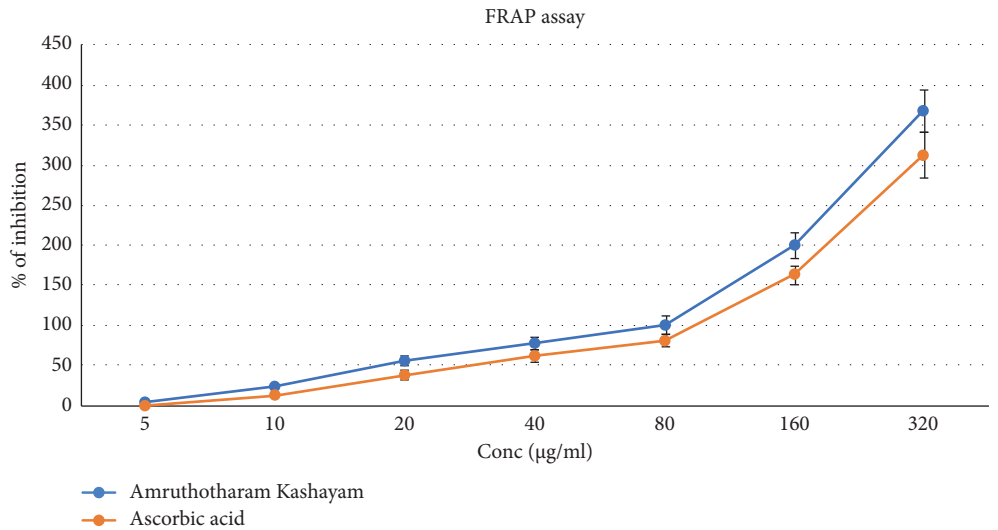

Figure 6: Comparative graphical representation of FRAP assay of Amritotharanam Kashyam and ascorbic acid.

fluctuation), and hydrogen bond plots were generated to evaluate the structural changes or stability in the complex. RMSD plot showed a slight deviation with the stability observed from 30 ns throughout the simulation period; the complex attained a slight deviation at 0.4 nm. RMSF plot showed less fluctuation in the loop or disorder region at the initial simulation period and 0.35 nm. These deviations do not affect the structural changes or stability of the complex. Hydrogen bond interaction between the protein and the ligand was noted as five. The overall result (Figure 11) concluded that the complex showed better stability without changes in structural confirmations.

4. Discussion

Phytochemical substances found in medicinal plants are a plentiful source of treatment for several chronic disorders. In recent years, many therapeutic plants have produced numerous powerful biomolecules. According to scientists, these powerful chemical components from nature are employed to cure various illnesses with fewer adverse effects [20]. Plant extracts may contribute to the performance and general well-being of the fowl. The enhancement of endogenous digestive enzyme production, activation of the immunological response, and antibacterial, antiviral, antioxidant, and antihelminthic properties are just a few of the positive effects of herbal extracts or active substances on poultry nutrition. Computational developments significantly influenced the process of developing new drugs. Virtual screening methods are often and widely used to reduce the price and duration of drug development. A key component of structure-based drug design is the discovery of new ligands for protein structures using the molecular docking technique [21]. 1, 2, 3-benzenetriol and 4YOI interacted with GLN 167 with docking score of −7.325. Interaction profile between the 1, 2, 3-benzenetriol and

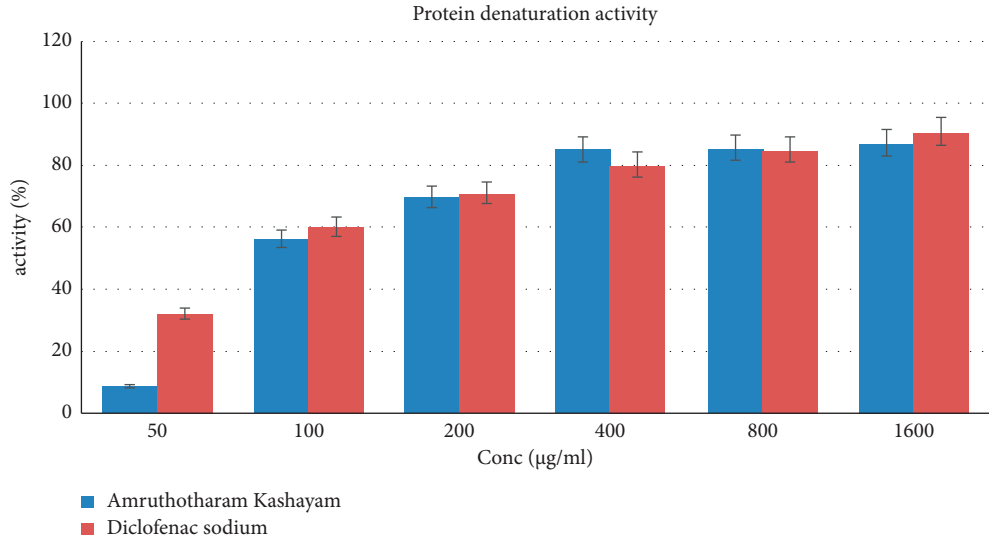

Figure 7: Comparative graphical representation of protein denaturation activity of Amritotharanam Kashyam and diclofenac sodium.

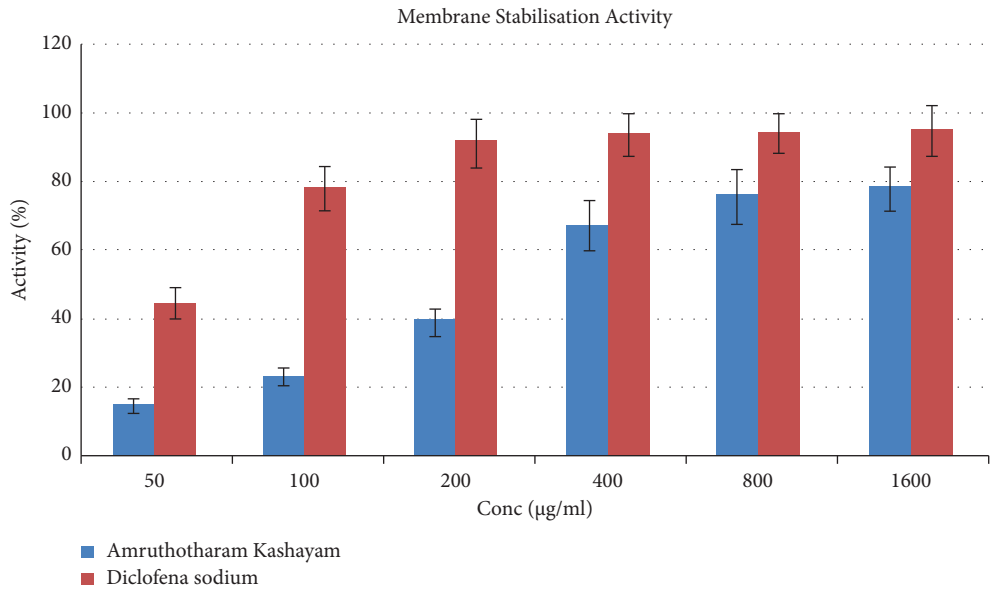

Figure 8: Comparative graphical representation of the membrane stabilisation activity of Amritotharanam Kashyam and diclofenac sodium.

6YB7 show the docking score of −5.621and interacting with GLU 166 amino acid. Interaction profile between the 1, 2, 3-benzenetriol and 6M17 show the docking score of −6.814 and interacting with LEU 52 and TYR128 amino acid. Targeted receptor 1M17 docked against the 1, 2, 3-benzenetriol and the interacting residues are GLU 738 and THR 766. 5DXT docked against 1, 2, 3-Benzenetriol reveals the interacting residue between the ligand and protein receptor such as VAL 851 and GLU 849. *In silico* approaches revealed the potential efficacy of the phyto-compounds against the target receptors of breast cancer and SARS-CoV 2. The resultant phytocompounds have potential therapeutic efficacy, which was analyzed *in vitro* and *in silico* examinations.

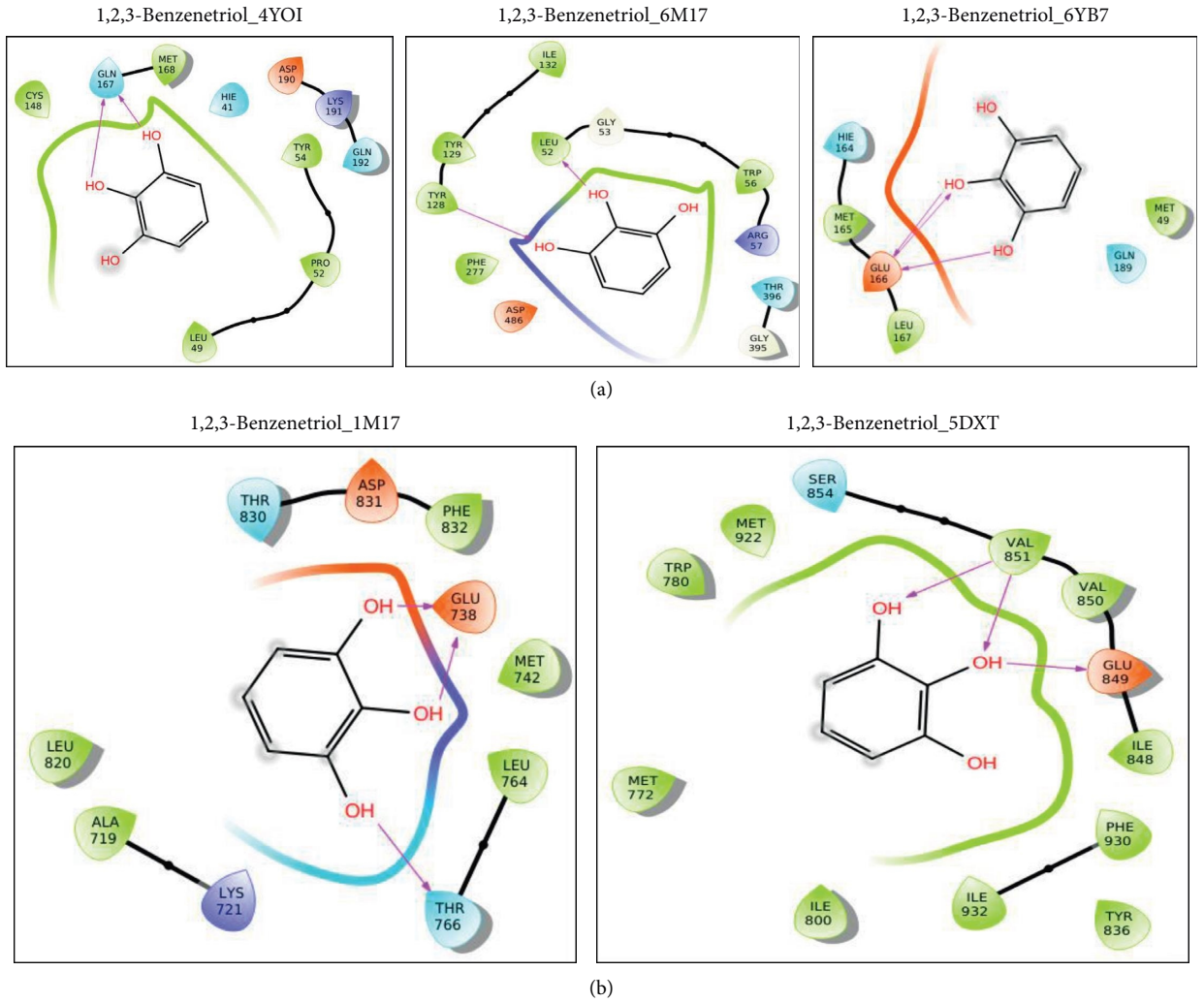

Figure 9: (a) Docking interactions of lead compound 1, 2, 3-Benzenetriol with the SARS-CoV-2 target proteins; (b) docking interactions of lead compound 1, 2, 3-Benzenetriol with the breast cancer target proteins.

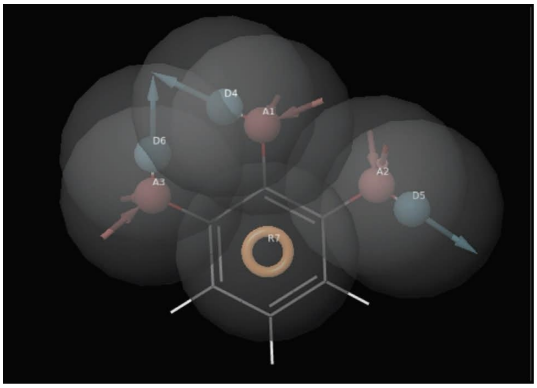

Figure 10: Pharmacophore hypothesis of 1, 2, 3-Benzenetriol.

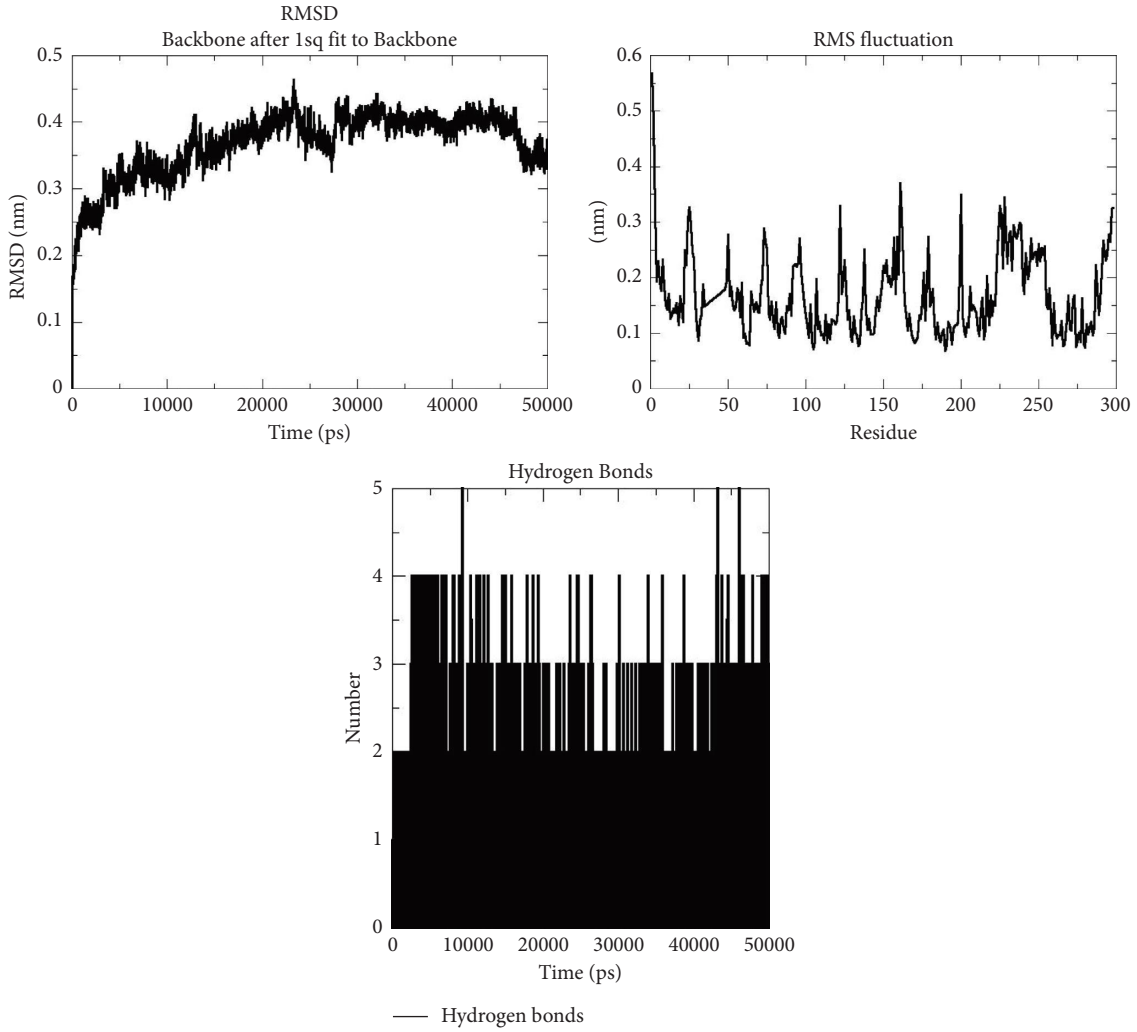

Figure 11: Molecular dynamics simulation on the lead complex 1, 2, 3-Benzenetriol_4YOI.

5. Conclusion

Overall, based on the findings of the tests mentioned above, it was evident that the compounds identified in the GC MS profile may aid in the function of this drug as a potent antioxidant and anticancer agent. Molecular docking and dynamics, ADME prediction, and pharmacophore hypothesis are some of the computational methods used in the current work to inhibit expression. According to a molecular dynamics simulation study, reveals the lead docked complex showed improved stability and reduced fluctuation with a significant number of hydrogen bond interactions. The phytocompounds' potential effectiveness against the SARS-CoV 2 and breast cancer target receptors was discovered using *in silico* approaches. Thus, these results indicate the genius of the complementary and alternative medicine practitioners.

Data Availability

All data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

LSR, PK, EP, and RKRM conceptualized the study. NKT, KL, and GS wrote the original draft. AS, SC, KC, and BRS supported in revision and editing the manuscript. SSK supervised the study. All authors have read and agreed to the published version of the manuscript. Langeswaran

Kulanthaivel, Anitha Shanmuganathan, Kirubhanand Chandrasekaran, Bharat Ramrao Sontakke and Gowtham Kumar Subbaraj contributed equally as first authors to this work.

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