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Polyphenolics Screening in Mollungo Nudicaulis Using Uplc-Esi-Ms and Its Active Compound Kaempferol against Sars Cov2 Receptor

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ABSTRACT
The present study was conducted to evaluate Mollungonudicaulispolyphenolics screening through LC-ESI-MS and its compounds drug molecular docking analysis and anti-oxidant capability analysis through invitro assays.Optimization of extraction was carried out using three different methods; among all methods the 90% ethanol using different solvents ratio,the total phenolics and flavonoid content was quantified through spectrophotometricallyand polyphenolicsscreeningthrough LC-MS in both positive and mode ionization.Total phenolics and flavonoid content of MN 90% ethanolic extract was quantified through spectrophotometrically (50 ± 0.85 and 48.52 mg/kg), Higher flavonoid content plays major role on antioxidant activities, which were evaluated and compared with commercial antioxidant butylatedhydroxytoluene (BHT) employing superoxide anion scavenging activity andtotal reducing power IC_{50} value results shows 152 and 135 µg/ml respectively.The extracted sample with known quantity (10µg/ml to 200µg/ml) used against E. coli, S. aureus and B. subtilis, MIC results shows 43.2± 3, 22 ± 2 and 21 ± 4 µg/ml respectively. The literature survey revealed that so far, no scientific studies carried out on phytochemical identification and confirmation using LC-MS analysis of Mollugonudicaulis leaves. Hence, in the present study, we focused to evaluate the phytochemical content, docking and antimicrobial ability of Mollugonudicaulis.Antimicrobial activity examined against E. coli, S. aureus and B. subtilis species using spectroscopically.Molecular docking analysis using 1-Click Docking software.conclude plant extract were act as good anti-oxidant and anti-microbial agent.

Keywords: Mollugonudicaulis, LC-ESI-MS, Anti-microbial and SARs Cov2

I. INTRODUCTION:

Exogenous and endogenous elements in the human body create free radicals. Superoxide anion (O2-), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO), and nitric oxide are the most frequent reactive oxygen species (ROS) (NO). ROS are involved in a variety of processes in cells, including energy synthesis, phagocytosis, and intercellular communication. These reactive oxygen species (ROS) are produced by sunlight, ultraviolet light, ionising radiation, chemical reactions, and metabolic processes, and they play a role in a wide range of metabolic diseases, including DNA damage, carcinogenesis, and degenerative diseases like cardiovascular disease, ageing, and neurodegenerative diseases, such as atherosclerosis and rheumatoid arthritis. Antioxidants are chemicals that have the capacity to bind to free radicals and trap them. Antioxidant chemicals can be manufactured (BHA and BHT) or natural (plant secondary metabolites such as polyphenols and flavonoids).Antioxidants scavenge free radicals including peroxide, hydroperoxide, and lipid peroxyl, inhibiting the oxidative pathways that cause degenerative illnesses [1, 2]. In recent decades, there has been a surge in interest in discovering naturally occurring antioxidants in foods or medicinal plants to replace synthetic antioxidants, which are being limited owing to side effects such as inflammation and carcinogenicity, among other things. Antioxidants in nature can protect the human body from free radicals and slow the progression of many chronic illnesses. Many prior studies have shown that there are a huge number of plants that may be utilised to treat illnesses, with reactive oxygen species and free radicals playing a key role [3]. Both antioxidant and antibacterial properties are crucial for medicinal plants [4- 6].Aromatic and medicinal plants have

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been shown to create bioactive chemicals that interact with other species in the environment to limit bacterial and fungal growth [7, 8]. Spices and herbs have been shown to have antimicrobial properties against food-borne pathogenic bacteria in several investigations [9, 10]. Anjali Rawani et al. [11] recently demonstrated that aqueous, chloroform, and methanolic extracts of Alternantheraphiloxeroides, Plumeriaobtusa, Polyalthiacerasoides, and Ixora acuminate inhibited human infections. Mollugonudicaulis is a member of the Molluginaceae family that thrives during the wet season. Mollugonudicaulis has been used to treat whooping cough and jaundice for centuries.In female albino rats, the ethanolic extract of MN showed anti-diabetic efficacy against alloxan-induced diabetics . According to the literature review, there have been no scientific investigations on the antioxidant and antibacterial properties of Mollugonudicaulis leaves.[12]

As a result, the current study focused on determining the total phenolics and flavonoids content, as well as the antibacterial and antioxidant abilities of Mollugonudicaulis in vitro.

II. MATERIALS AND METHODS

Determination of total phenolics

The modified Folin-Ciocalteu technique [15] was used to quantify the total phenolic content of MN aqueous and methanolic extracts. To 100 litres of plant extract solution (1 mg/ml), 100 litres of Folin-Ciocalteu reagent and 200 litres of Na2CO3 (2 percent w/v) were added. The resultant mixture was incubated for 15 minutes at 45L with 120 rpm stirring. Using a uv-visible spectrophotometer, the absorbances of the samples were measured at 765 nm. The results are given in milligrammes of gallic acid equivalent per gramme of plant extract. The same process was used to create a standard curve using gallic acid, with concentrations ranging from 0 to 10 g/ml.

Result - 50 ± 0.85

2.2. Estimation of total flavonoids

The content of total flavonoids was calculated using the Aluminum chloride technique [16]. One millilitre of sample (1 mg/mL) was combined with 3 millilitres of methanol, 0.2 millilitres of 10% aluminium chloride, 0.2 millilitres of 1 M potassium acetate, and 5.6 millilitres of distilled water, and maintained at room temperature for 30 minutes. A UV-Visible spectrophotometer was used to measure the absorbance of the reaction mixture at 420 nm. The total flavonoid content was calculated using a rutin calibration curve (0-100 g/mL in methanol) as a reference. Total flavonoids were measured in milligrammes of rutin equivalent per gramme of plant extract.

Result - 48.52 mg/kg

Determination of reducing power

The reducing power was calculated using the method reported in [19, 20]. To 1.0 ml of the extract diluted in distilled water, 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of K3Fe(CN)6 (1 percent w/v) were added. The resultant mixture was then incubated at 50°C for 20 minutes before adding 2.5 mL of TCA (10% w/v). The top layer of the solution (2.5 ml) was collected after centrifugation at 5000 rpm for 10 minutes, then mixed with distilled water (2.5 ml) and 0.5 ml FeCl3 (0.1 percent, w/v). At 700 nm, the absorbance was measured against a blank sample. The results are shown in fig 1 and table 1.

Result: total reducing power IC_{50} value results shows 135 µg/ml

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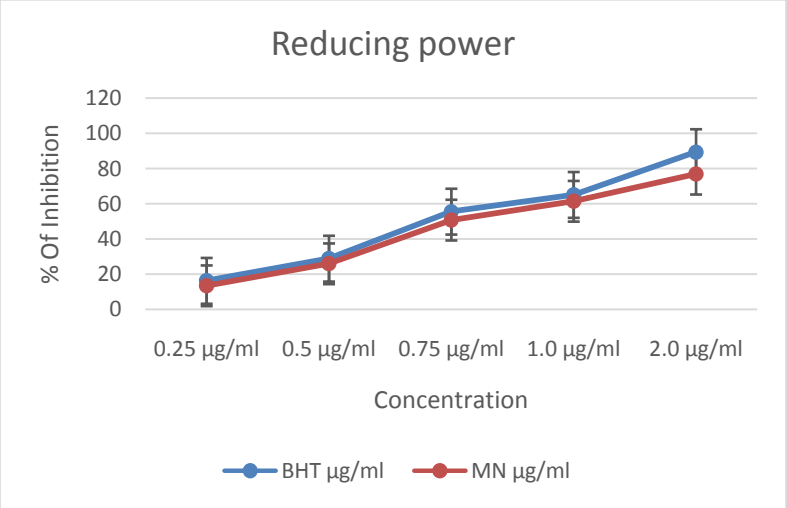

Figure 1: graphical representation ofReducing power of BHT and MN

Table 1: table representation of BHT and MN

Reducing power	0.25 µg/ml	0.5 µg/ml	0.75 µg/ml	1.0 µg/ml	2.0 µg/ml
BHT µg/ml	16.3	28.9	55.6	65.1	89.4
MN µg/ml	13.5	26	50.8	61.5	76.9

Superoxide anion scavenging

The superoxide anion scavenging activity of the extract was determined by the method of Yen and Chen [25]. The reaction mixture, consisting of 1 ml NBT, 1 ml of plant extract (20–100 g/ml), 1 ml of 60 M PMS (prepared in 0.1 M phosphate buffer, pH 7.4) and 1 ml of NADH (in phosphate buffer), was incubated at 25 ◦C for 5 min, and the absorbance was read at 560 nm. The percentage of scavenging inhibition of superoxide radicals was calculated from the equation in Section 2.8.the results where shown in fig 2 and table 2.

DPPH scavengingactivity(%) = (Ac − As) (Ac) × 100 where

Ac is the absorbance of DPPH + methanol, and As is the absorbance of free radical + sample (i.e., standard or plant extract).

Result : Superoxide anion scavenging activity IC_{50} value 152µg/ml

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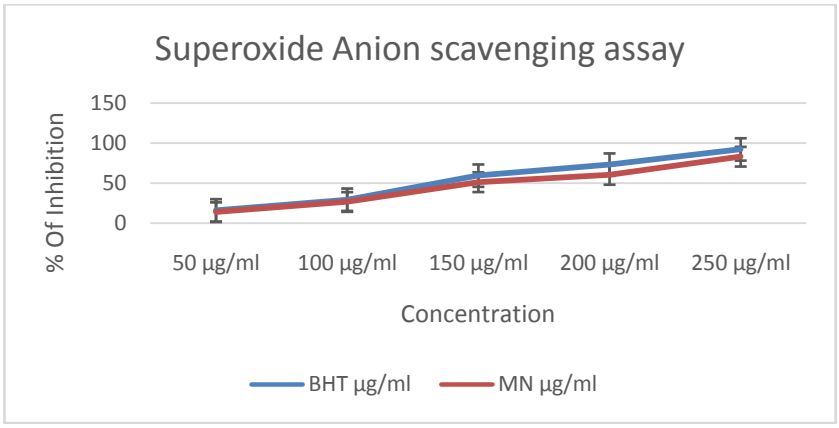

Figure 2: Graphical representation of Superoxide Anion scavenging assay of BHM and MN

Table 2: table representation of Superoxide Anion scavenging assay of BHM and MN

Superoxide anion	**50 µg/ml**	**100 µg/ml**	**150 µg/ml**	**200 µg/ml**	**250 µg/ml**
BHT µg/ml	16.1	29.4	59.5	73.3	92.3
MN µg/ml	13.8	26.6	51.3	60.5	83.2

RP-HPLC

Optimization of RP-HPLC methodThe analytical HPLC system employed consists of high performanceliquid chromatography (Waters, USA) coupled with a photodiodearray detector (PDA-2998, USA). A C18 reverse phase column of4.6×250 mm, 5 µm particle size (SYMMETRY) was used. The mobilephase used was water with 0.1% formic acid as solvent A and 100%ethanol as solvent B. The different isocratic and gradient programswere followed for phenolic compound separation. The optimized gradientprogram was 0–10% B (5 min), 10–15% B (5 min), 15–20% B (5 min), 20–80% B (5 min), with 1.0 ml/min as the flow rate, and 20 µl as the injection volume. The monitoringwavelength was detected between 210 and 400 nm. The analyticaldata was evaluated using EMPOWER 2 data processing softwarefrom Waters (Rameshkumar, Sivasudha, Jeyadevi, Arul Ananth, &Pradeepha,2012). Fractions were collected for each peak isolated in HPLC. The obtained graph is shown in fig 3.

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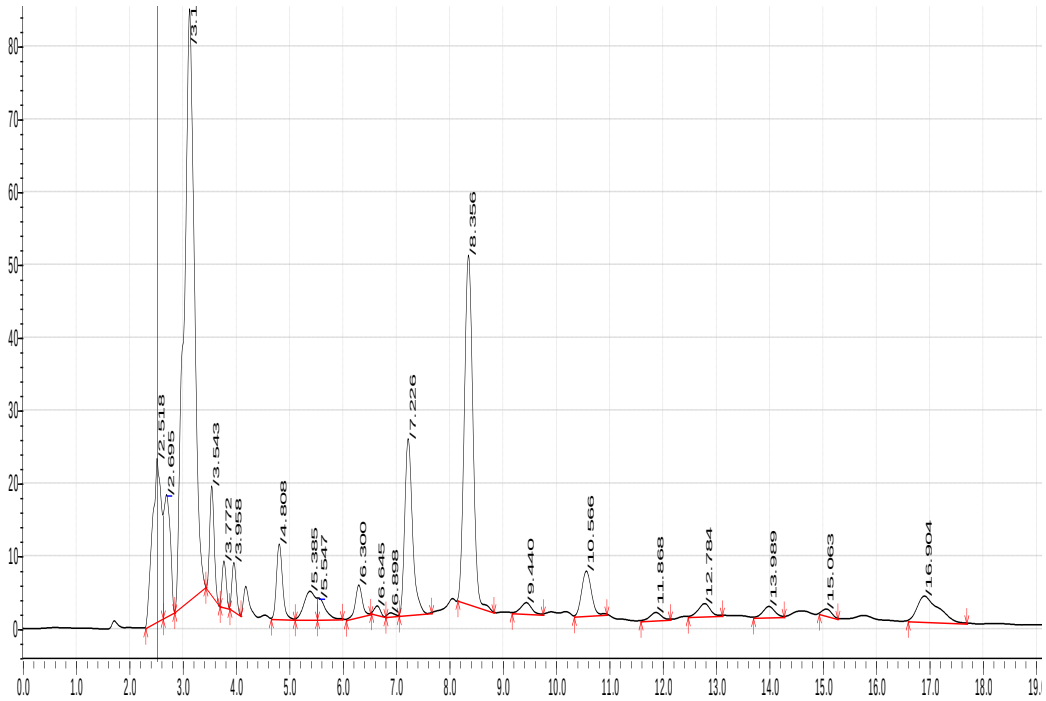

Figure 3: graph of HPLC

ESI-MS

UPLC–MS analyses were carried out using an ultra performanceliquid chromatography apparatus equipped with PDA detector(Waters, USA).Each fraction collected from HPLC was directly injected in ESI-MS, direct infushion method followed in MS the graph was shown in fig 4. Mass spectroscopicanalysis of phenolic compounds in the sample was performed using aSYNAPT mass spectrometer (Waters), equipped with an electrosprayionization source operating in negative ion modes, mass range 100 to 2000 was used in scan method.Phenolic compounds of MNleaves were tentatively characterizedby comparison with their UV–vis absorption spectra and comparison of MSm/z with reference standardsand/or literature reports. The identified phenolic compounds are listed in table 3.

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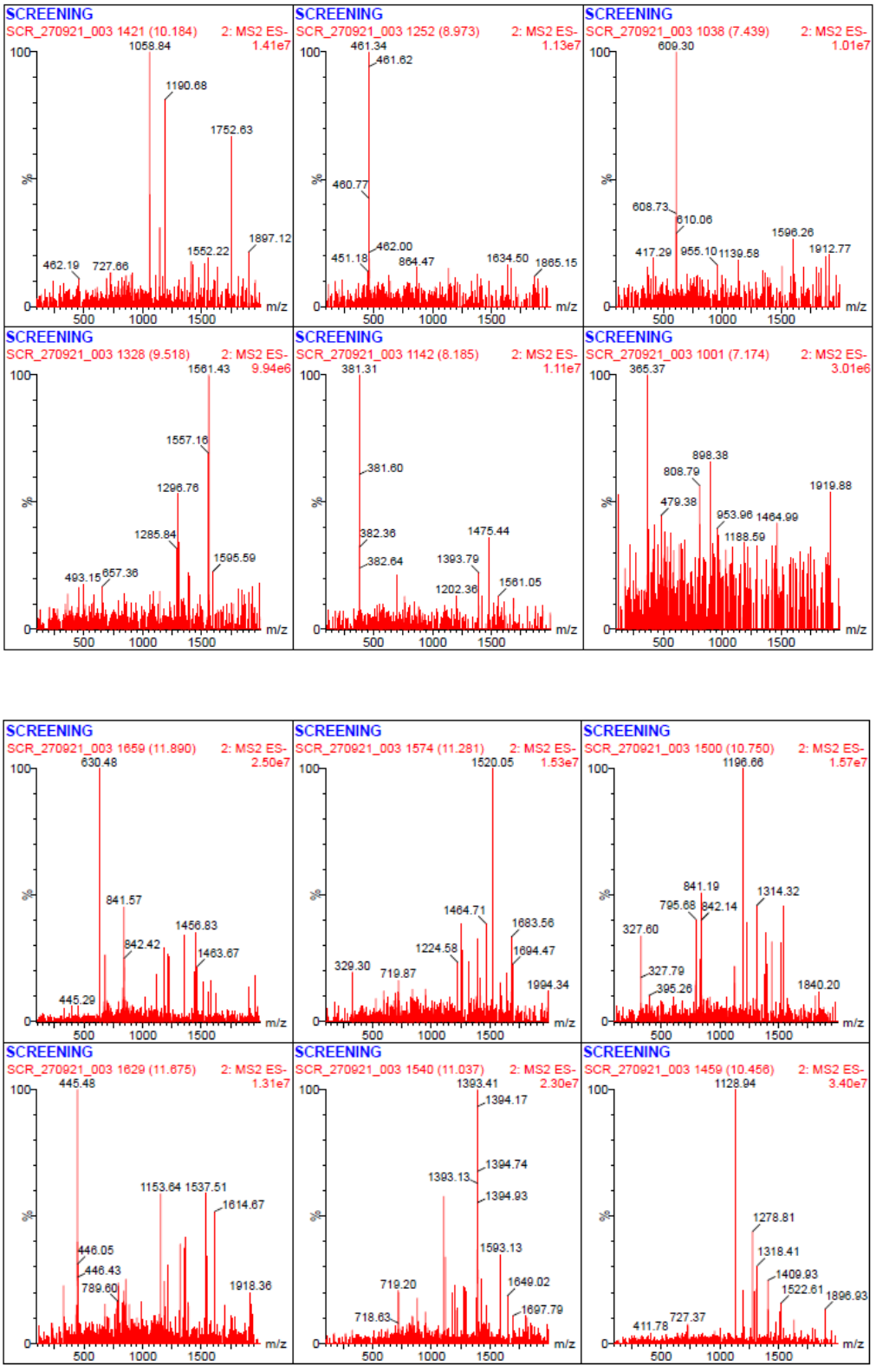


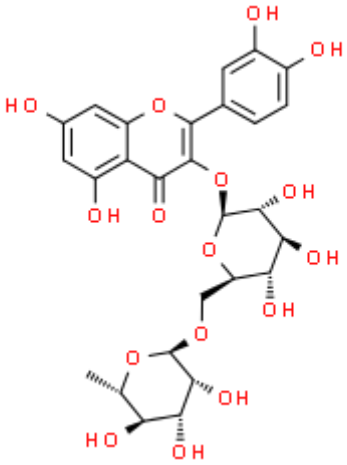
Figure 4: graph obtained by UPLC–MS

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 2 Mar-Apr 2022, pp: 297-309www.ijprajournal.com ISSN: 2456-4494

Table 3: list of phenolic compouns identified in MN leaves.

S.No	RT	Mode [M-H]	Ion (m/z)	Compound	Reference
1	7.174	_	365.37	Unknown	
2	8.18	_	381.31	Unknown	
3	7.439	_	609.3	Rutin	Sayed et al, 2020
4	8.973	_	461.34	Kaempferol-3-O-Glucuronide	Sayed et al, 2020
5	9.518	_	1561	Unknown	
6	10.18	_	1058.8	Unknown	
7	11.48	_	630	Unknown	
8	10.45	_	1128.9	Unknown	
9	10.75	_	1196	Unknown	
10	11.037	_	1393	Unknown	
11	11.28	_	1520	Unknown	
12	11.67	_	445.48	Physcion 8-O-glucoside	Chen et al 2016
13	11.89	_	630.48	Unknown	

Rutin



Kaempferol 3-O-glucuronide

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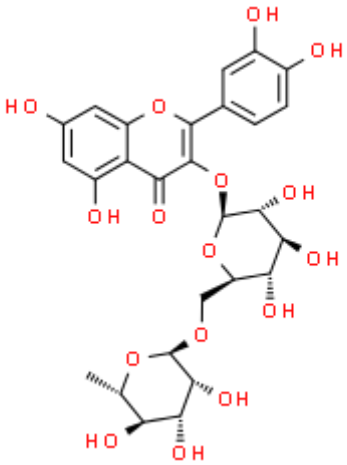
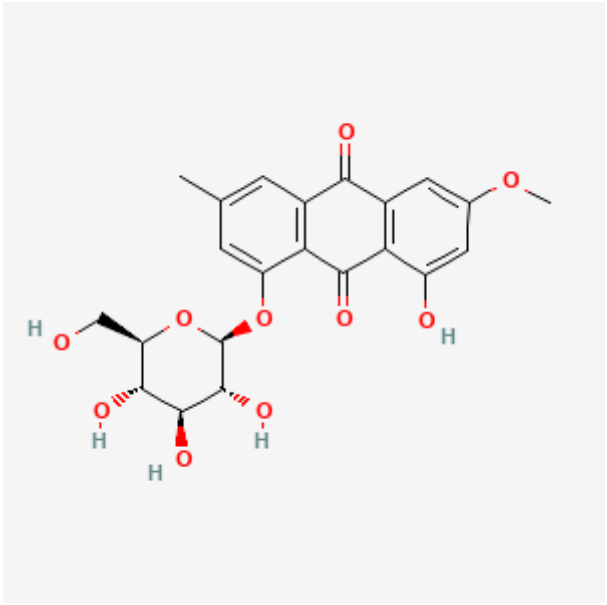


Physcion 8-O-glucoside



Minimum Inhibitory Concentration

The antimicrobial activity was carried out using minimum inhibitory concentration method [2]. Samples were diluted in distilled water with 10% of dimethyl sulfoxide DMSO because of solubility of flavonoids. 100μL of fruit sample solution was added to the sterile 96 well plate containing 100μL media (Mueller-Hinton) and 100μL of bacterial species (E-coli (ATCC25922), S. aureus (CMCC(B)26003) and B. subtilis (MTTC N0-10110))used as reference material.The microbial suspension was mixed and absorbance were calculated. Then cultures were incubated 37°C for 24 hours and the absorbance were monitored. The without sample incubated microbial plates are used as control sample

Results :E. coli, S. aureus and B. subtilis, MIC results shows 43.2± 3, 22 ± 2 and 21 ± 4 µg/ml respectively.

Molecular Docking of Rutin against breast cancer line MDA-MB-231 receptor

To give the proof for our concept that quercetin could bea potential drug against novel coronavirus, we havefollowed the molecular docking methodology. Threedimensionalstructures of 2GZ8 proteins of coronavirus were retrieved. 2GZ8-Structure-Based Drug Design and Structural Biology Study of Novel Nonpeptide Inhibitors of

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Volume 7, Issue 2 Mar-Apr 2022, pp: 297-309www.ijprajournal.com ISSN: 2456-4494

SARS-CoV Main Protease. The structure was shown in figure 5.

2GZ8 Protease Structure (Reference: RCSB.Org)

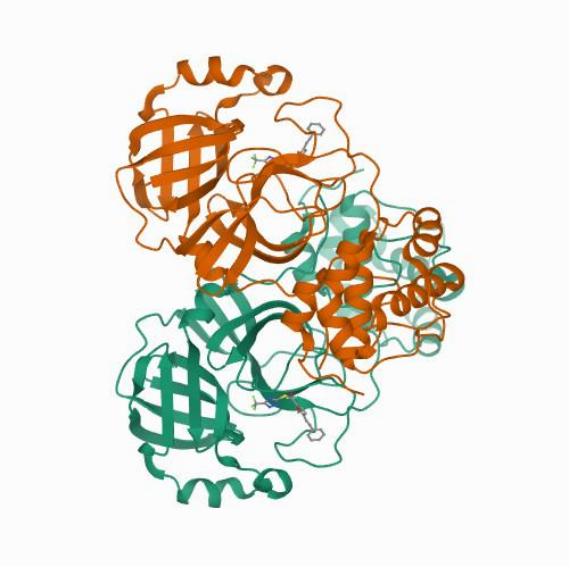

Figure 5:protease structure

Results for Docking

The active sites of the proteins wereidentified using 1-click docking. The 3D structures ofthe proteins and the ligand were loaded into moleculardocking software. The active sites identified were provided below, and the docking grid was setaccordingly and the docking was performed. The obtained result was shown in figure6.

Docking pose	Docking score		
#1	-8.0	VISUALIZE POSE	DOWNLOAD POSE
#2	-7.2	VISUALIZE POSE	DOWNLOAD POSE
#3	-7.1	VISUALIZE POSE	DOWNLOAD POSE
#4	-6.1	VISUALIZE POSE	DOWNLOAD POSE

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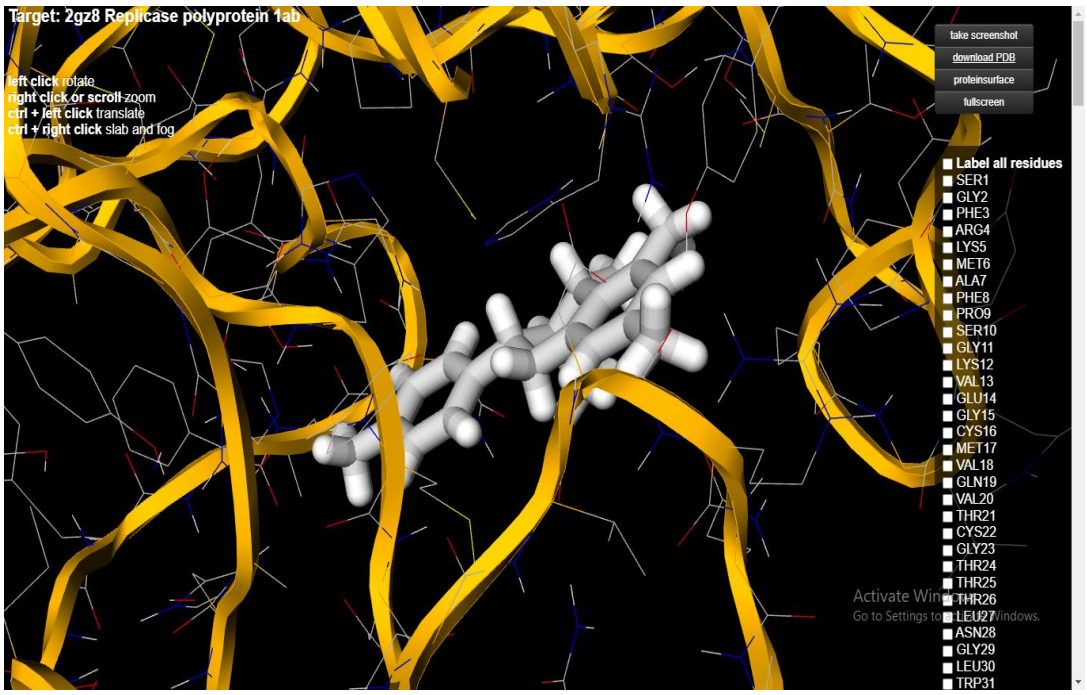

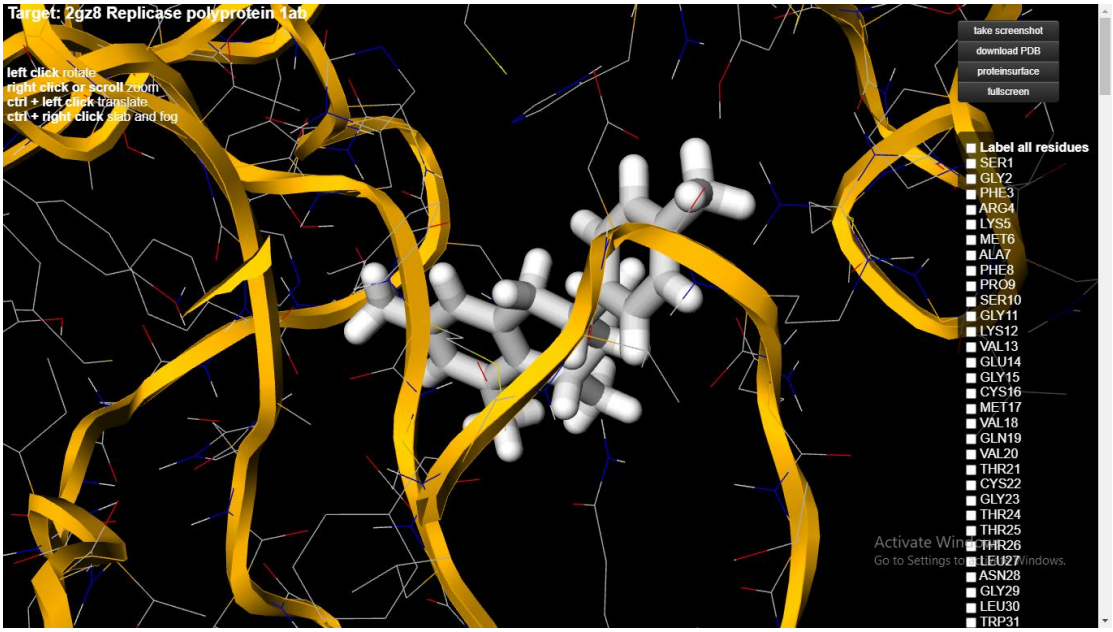

International Journal of Pharmaceutical Research and Applications
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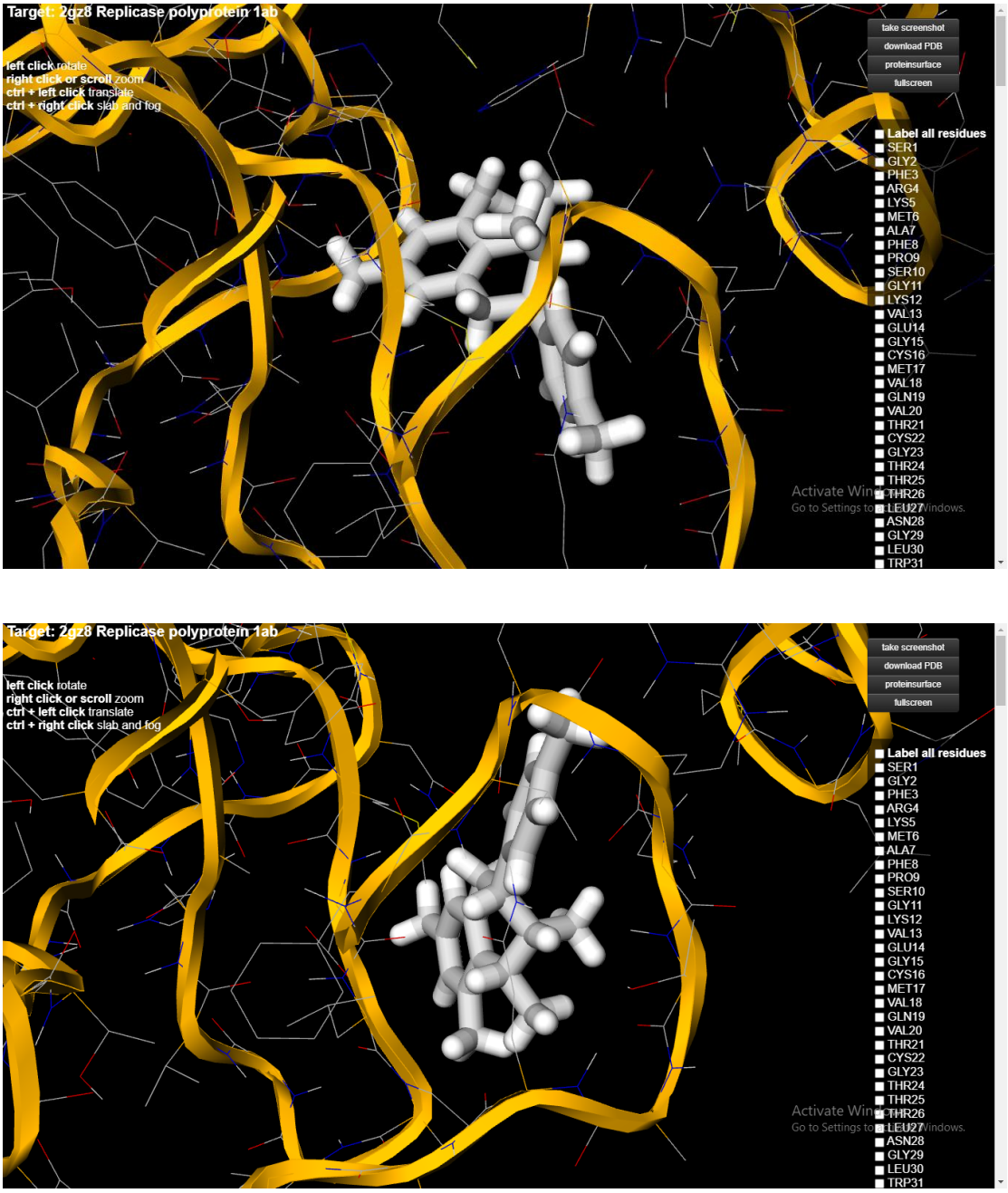

Figure 6:results of molecular docking, The 3D structures of the proteins and the ligand

International Journal of Pharmaceutical Research and Applications
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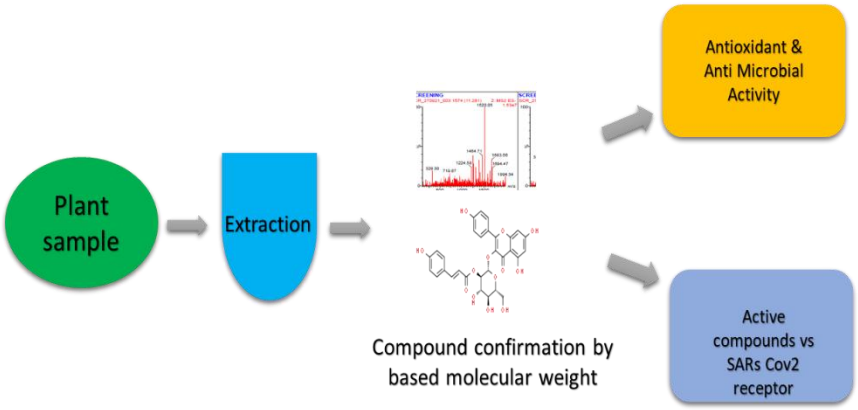
Overview of the Study



III. CONCLUSION:

The maximum yield of phenolics and flavonoids content shows method suitability of flavonoids extraction and quantification. Further, plant extract versus anti-oxidant and its role against SARs Cov2 receptor. Further, the receptor binding possibilitis was confirmed using molecular docking study against kaempferol.Results conclude plant extract were act as good anti-oxidant and anti-microbial agent.

IV. ACKNOWLEDGEMENT:

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