POLYPHENOLICS SCREENING IN *MOLLUNGO NUDICAULIS* USING UPLC-ESI-MS AND ITS ACTIVE COMPOUND KAEMPFEROL AGAINST SARs CoV2 RECEPTOR

A Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY,

CHENNAI- 600 032

In partial fulfilment of the award of the degree of

MASTER OF PHARMACY

IN

Branch-II -- PHARMACEUTICAL CHEMISTRY

Submitted by

Name: KANMANI R

REG.No. 261915205

Under the Guidance of

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J.K.K.NATTARAJA COLLEGE OF PHARMACY
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TAMILNADU.

OCTOBER – 2021

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CERTIFICATES

EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"POLYPHENOLICS SCREENING IN *MOLLUNGO NUDICAULIS* USING UPLC-ESI-MS AND ITS ACTIVE COMPOUND KAEMPFEROL AGAINST SARsCoV2 RECEPTOR" ,** submitted by the student bearing **Reg. No: 261915205** to "**The Tamil Nadu Dr. M.G.R. Medical University – Chennai**", in partial fulfilment for the award of Degree of **Master of Pharmacy** in **Pharmaceutical Chemistry** was evaluated by us during the examination held on…….

Internal Examiner **External Examiner**

CERTIFICATE

This is to certify that the work embodied in this dissertation entitled**, "POLYPHENOLICS SCREENING IN *MOLLUNGO NUDICAULIS* USING UPLC-ESI-MS AND ITS ACTIVE COMPOUND KAEMPFEROL AGAINST SARsCoV2 RECEPTOR"** submitted to "**The Tamil Nadu Dr. M.G.R. Medical University- Chennai**", in partial fulfilment and requirement of university rules and regulation for the award of Degree of **Master of Pharmacy** in **Pharmaceutical Chemistry** , is a bonafide work carried out by the student bearing Reg.No**. 261915205** during the academic year 2020-2021, under the guidance and supervision of **Dr.M.SENTHIL RAJA, M.Pharm.,Ph.D.,**Professor,Department of Pharmaceutical Chemistry, J.K.K.Nattraja College of Pharmacy, Kumarapalayam.

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I do hereby declared that the dissertation**"POLYPHENOLICS SCREENING IN *MOLLUNGO NUDICAULIS* USING UPLC-ESI-MS AND ITS ACTIVE COMPOUND KAEMPFEROL AGAINST SARsCoV2 RECEPTOR"** submitted to "**The Tamil Nadu Dr. M.G.R Medical University - Chennai",** for the partial fulfilment of the degree of **Master of Pharmacy** in **Pharmaceutical Chemistry ,** is a bonafide research work has been carried out by me during the academic year 2020-2021, under the guidance and supervision of **Dr. M.SENTHIL RAJA, M.Pharm., Ph.D.,** Professor, Department of Pharmaceutical Chemistry , J.K.K. Nattraja College of Pharmacy, Kumarapalayam.

I further declare that this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma, associate ship and fellowship or any other similar title. The information furnished in this dissertation is genuine to the best of my knowledge.

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SIGNATURE OF THE CANDIDATE

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Abbreviations

BHT - butylated hydroxytoluene

LC-MS - Liquid chromatography–mass spectrometry

MICs - Minimum inhibitory concentrations

SARs - Severe acute respiratory syndrome

MN - Mollungo nudicaulis

ESI – Electron spray ionization

ROS - Reactive Oxygen Species

H_2O_2 - hydrogen peroxide

ROO - peroxyl radicals

NO - nitric oxide

LC–DAD - liquid chromatography with diode array detector

DPPH - 2,2-diphenyl-1-picrylhydrazyl

ABTS - 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

MRM - multiple reactions monitoring

HPLC- High pressure liquid chromatography

SO2- - superoxide anion

H_2O_2 - hydrogen peroxide

ROO - peroxyl radicals

NO - nitric oxide

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COLLEGE OF ARTS & SCIENCE

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Based upon the organoleptic / macroscopic / microscopic examination of fresh sample, it is certified that the specimen given by **Mrs.R Kanmani,** Final year, M.Pharm, JKK Natraj College of Pharmacy, Kumarapalayam, Erode-638183.Is identified as below

Bionomial	: ***Mollugo nudicaulis Lam***
Family	: Molluganaceae
Synonym(s)	: *Lampetia nudicaulis(Lam)*
Regional names	: Nacked – stem carpetweed (English) and Kuthuthirai (Tamil)

Place:

Date: 10-02-2022

Authenticated by

10/02/22

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INTRODUCTION

1. INTRODUCTION

Free radicals are produced by exogenous and endogenous factors in the human body. The most common reactive oxygen species (ROS) includes superoxide anion (O2-), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO) and nitric oxide (NO). ROS play an important role in cell metabolism including energy production, phagocytosis and intercellular signalling. These ROS produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes have a role in wide variety of metabolic diseases such as DNA damage, carcinogenesis and various degenerative disorders such as cardiovascular diseases, aging and neuro-degenerative diseases, atherosclerosis and rheumatoid arthritis. Antioxidants are the compounds which has the ability to trap free radicals.

Antioxidant compounds could be either synthetic (BHA and BHT etc) or natural (plant secondary metabolites such as polyphenols and flavonoids). Antioxidants scavenge the free radicals, such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [1, 2.] Recent decades, the interest has increased considerably in finding naturally occurring antioxidants in foods or medicinal plants to replace synthetic antioxidants, which are being restricted due to their side effects such as inflammation and carcinogenicity etc. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases.

Many of the previous literatures show large number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role [3]. Medicinal plants are playing an important role in both antioxidant and antimicrobial activities [4- 6]. Aromatic

and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth [7, 8]. Many studies reported the activities of spices and herbs on food borne pathogenic microorganisms [9, 10]. In the recent investigation, Anjali Rawani et al [11] reported that the aqueous, chloroform and methanolic extracts of Alternanthera philoxeroides, Plumeria obtusa, Polyalthia cerasoides and Ixora acuminate showed inhibition against human pathogens. Mollugo nudicaulis belongs to the family Molluginaceae which is prevalent commonly during the rainy season. Mollugo nudicaulis is traditionally used to treat whooping cough and jaundice. The ethanolic extract of MN shown anti-diabetic activity against alloxan induced diabetics in female albino rats [12]. The literature survey revealed that so far, no scientific studies carried out on antioxidant and antimicrobial capabilities of *Mollugo nudicaulis* leaves. Hence, in the present study, we focused to evaluate the total phenolics and flavonoids content, invitro anti-oxidant and antibacterial ability of *Mollugo nudicaulis*.

Flavonoids are natural pigments present in the plant or microbial sources and correspond to a specific group of chemical constituents called phenolic compounds [1,2]. They are found in vegetables, seeds, fruit, and various fruits and alcoholic beverages [3]. Flavonoids have important positive effects on human health especially due to their antioxidant and free radical scavenging. Although several studies have shown that some flavonoids have a pro-oxidant effect, they only occur at high doses, most of which confirm the existence of anti-inflammatory effects, antiviral or anti-allergic, and their protective role against cardiovascular disease, cancer, and various pathologies [2,4]. Flavonoids protect the human body from damage caused by oxidizing agents such as ultraviolet rays, environmental pollution, food chemicals, etc.

The human organism cannot produce these chemicals in a protective manner, so they must be obtained by means of feed or as supplements. These compounds have been discovered by Nobel Prize winner Szent-György, who in 1930 isolated asubstance, citrine, which regulates the permeability of the capillaries from the lemon peels. Flavonoids were first identified as vitamin P (because of the ability to increase capillary permeability) and vitamin C2 (because some flavonoids had similar properties to vitamin C) [1.] However, the fact that flavonoids were vitamins could not be confirmed, and both names remain around 1950. Flavonoids contain in their chemical structure a variable number of phenolic hydroxyl groups and excellent properties of iron chelation and other transition metals, which give them a high antioxidant capacity; therefore, they play an essential role in the protection against oxidative damage and have therapeutic effects in a wide range of conditions, including heart disease ischemic, or atherosclerosis cancer [5–7].

Anti-free radical properties of flavonoids are primarily aimed at hydroxyl and superoxide radicals, highly reactive species involved in the onset of lipid chain peroxidation and described their ability to modify eicosanoid synthesis (with anti-prostanoid and anti-inflammatory reactions) to prevent platelet aggregation (antithrombotic effects) and to protect low-density lipoproteins from oxidation [8,9]. In addition to its known antioxidant effects, flavonoids have other properties, including stimulation of communication through gap junctions, effects on the regulation of cell growth and induction of enzymes, detoxification such as dependent monooxygenase Cytochrome P-450, among others. [10]. However, most of the biological properties of flavonoids are strongly determined by the mode of extraction for their recovery. Efforts have recently been reported to develop biotechnological strategies to reduce or eliminate the use of toxic solvents, reduce processing time, and maintain the bioactivity of the compounds.

Flavonoids are a type of polyphenolic compound, its chemical structure is varied but the general skeleton structure is composed of 15 carbones (C6-C3-C6), which are grouped in two aromatic rings (A and B) connected by a 3-carbon bridge that gives rise to an oxygenated heterocycle (C) [11–15]. Flavonoids are derivatives of 1, 3-diphenylpropan-1-one and their biosynthetic pathway is the condensation of three malonyl-CoA molecules with one p-coumaroyl-CoA molecule to the intermediate chalcone [16,17]. Flavonoids are water-soluble pigments present in the plant kingdom as secondary plant metabolites [2,18,19], which can be found specifically in the cytosol and stored in the plant cell vacuole.

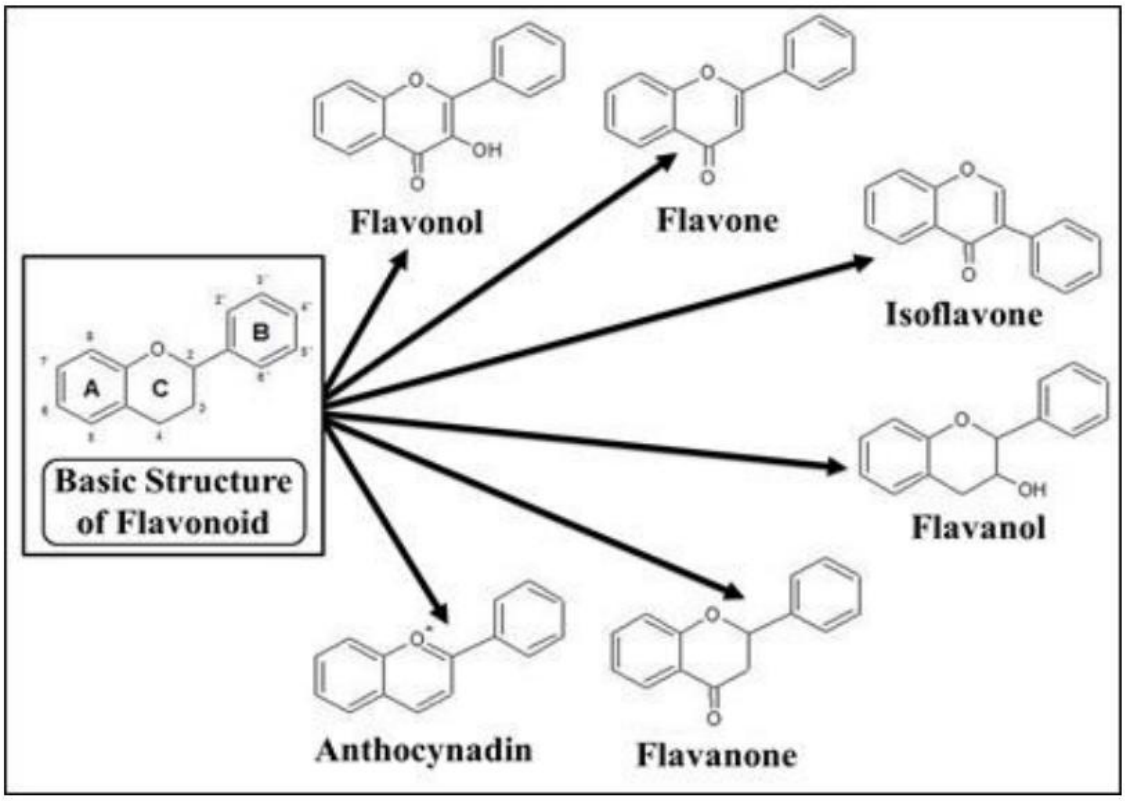


Figure 1. Basic chemical structures of flavonoids and their different class.

Flavonoids are classified according to differences in the structure of the heterocyclic C ring; these differences may be caused by the oxidation state and the degree of unsaturation of the heterocyclic ring (or the lack thereof in the case of chalcones). It has been estimated that the number of identified flavonoids exceeds 7000 and that the number of flavonoids continues to increase due to their important biological activities [17]. Variations in the basic structure of flavonoids give rise to six different classes of this group of compounds (Figure 1): Isoflavones, flavanone, flavanone, flavan-3-ol, flavonol, and anthocyanidin, each of which has particular characteristics [18,20].

Flavanones

Flavanones (dihydroflavones) have a structure that differs in the lack of a double bond (C2-C3) in the C-ring of the flavonoid structure. This type of flavonoid can be found in aromatic plants (such as mint), tomatoes, citrus (especially grapefruit) [12]. Flavanones can be found in nature as forms of aglycones and glycosides, some examples of such compounds are naringenin, hesperetin, and eriodictyol [21].

Flavonols

Flavonols are called 3-hydroxyflavones and are the most commonly found flavonoids in the plant kingdom [22]. A double bond between C2 and C3 and a hydroxyl group is included in their structures [13]. Some of the most important phytochemical compounds that represent this group are as follows: Myricetin, quercetin, isorhamnetin, and kaempferol [12,22,23]. They can be

found in a variety of colors (from white to yellow). In nature, flavonols can be found in two forms: Glycosides and aglycone (quercetin and kaempferol) [12].

Flavones

Flavones can be found in all parts of the plants, above-and belowground, in vegetative and generative organs; stem, leaves, buds, bark, heartwood, thorns, roots, rhizomes, flowers, farina, fruit, seeds, and also in root and leaf exudates or resin. They result from the introduction of a double bond between C2 and C3 by the abstraction of two hydrogen atoms [16,18]. Flavones are present in all major land-plant lineages. The plant species that contain flavones belong to over 70 different families in the plant kingdom [16].

Anthocyanins

Anthocyanins are primarily found in nature in glycosidic form. This type of flavonoid is responsible for plant pigment (such as blue, red, pink, and purple) by the formation of weak covalent bonding complexes with other organic compounds [23,24]. More than 500 anthocyanins have been reported and are the product of methoxylation, hydroxylation, and glycosylation patterns in the B ring. The most representative compounds of this subclass of flavonoids are pelargonidin, cyanidin, and delphinidin [12].

Flavanols

Flavanols (flavan-3-ols) are also called catechins, which have a typical flavonoid structure but have different hydroxylation patterns of rings A and B and asymmetrical carbon stereochemistry of ring C (C2 and C3) [25,26]. The catechins are classified into two groups; free catechins and esterified catechins [27], and constitute the most complex class of flavonoids due to

their size, monomers (catechin), or polymeric forms (condensed tannins) [21]. They can be found as the main ingredient in green tea [12].

Isoflavones

Another type of flavonoids, isoflavones are commonly referred to as phytoestrogens due to their considerable estrogen activity. They are characterized by the fusion of their ring B with the C3 position of the ring C [21,28–30]. They are an important group in a variety of fields, such as medicine, cosmetics, and nutrition. These flavonoids can be found in plants of the Leguminosae family (soybeans, alfalfa sprouts, and red clover leaves) [30,31]. Isolation and Extraction Methods There is a general methodology consisting of three stages for the isolation, extraction, and identification of phytochemicals from natural sources. Pre-treatment or preparation of a sample is the first step in which the centrifuge, filtration, or drying process and others can be used. In the second stage, the extraction, isolation, and purification of flavonoid compounds from different plant samples are most notably. In this step, phytochemicals are extracted using processes such as soxhlet, maceration, water infusion, supercritical fluid extraction, solid microphase extraction, microwave extraction, ultrasound, autohydrolysis, etc. In the last step, the purified and extracted extracts are normally used for further study by chromatography techniques, usually involving the identification, quantification, and recovery of flavonoid compounds.

Details of each method, such as conventional and emerging methods used by a number of researchers for flavonoid extraction, are given in the following sections: Conventional Methods Flavonoid extraction and recovery have been booming over recent years because of population trends in healthier lifestyles and the integration of antioxidants into the diet. Therefore, several methods for extracting flavonoids to increase the extraction yields of these major bioactive

compounds have been implemented. Various extractive methods have been proposed, including maceration, percolation, hydro-distillation, boiling, reflux, soaking, and soxhlet [32]. Soxhlet was the most commonly used method for the extraction of flavonoids due to its simplicity and ease of maintenance, low cost, and lower solvent content compared to other methods such as soaking, boiling, or maceration [14,33,34]. Various solvents such as ethanol, methanol, benzene, chloroform, ethyl acetate, etc. have been tested in this extraction method to compare the effect on extraction yields [15,32].

In general, liquid–liquid or solid–liquid extraction is the most widely used process for the extraction of flavonoids. Although maceration and water infusion are conventional extraction processes, they are still used today [35,36]. These methodologies have adopted the use of solvents such as ethanol, methanol, acetone and not just water for the extraction of bioactive compounds [14,37,38]. These conventional extraction methods are characterized by the use of large amounts of solvent, lower extraction yields, and long extraction times compared to other methods. It has been reported that when extraction methodologies involve heat treatments, degradation in the chemical structures of the extracted flavonoids can result in a reduction in bioactivity [39]. Parameters such as time, particle size, type of solvents, mass to volume ratio, temperature, etc. have been evaluated in conventional extraction methods of flavonoid (Table 1) [40–42]. The nature of the extracting agent (solvent) will affect the type of flavonoid extracted and will directly influence the biological activity of the recovered compounds. Of the solvents tested, ethanol and methanol are the most widely used for the extraction of flavonoids due to higher yields achieved in the recovery of flavonoids [43,44].

Quercetin is an important flavonol among the members of six subclasses of flavonoid compounds. The name quercetin was derived from quercetum (after Quercus, i.e., oak), and has been used since 1857. It has been named as 3,3′,4′,5,7- pentahydroxyflavone by the International Union of Pure and Applied Chemistry (IUPAC). It is also known by its synonym 3,3′,4′,5,7-pentahydroxy-2-phenylchromen-4-one [25]. Quercetin is the most widely distributed and extensively studied flavonoid found in various food sources, including fruits, vegetables, nuts, wine, and seeds. Quercetin has various biological properties, including antioxidant, anti-inflammatory, antibacterial, antiviral, radical-scavenging, gastro protective, and immune-modulatory activities [28]. In addition, in several recently-filed patents the wide therapeutic applications of quercetin and its derivatives have been described in detail. Quercetin exhibits a wide range of biological activities and therapeutic applications, which are of interest to the pharmaceutical, cosmetic, and food industries.

Medicinal and aromatic plants are the natural sources to treat various human diseases. Medicinal plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals [24]. The World Health Organization (WHO) estimates that 80 percent of the populations of some Asian and African countries presently use herbal medicine for some aspects of primary health care. Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Antioxidants are found in many foods, including fruits and vegetables. They are also available as dietary supplements. Vegetables and fruits are rich sources of antioxidants. There is good evidence that eating a diet with lots of vegetables and fruits is healthy and lowers risks of certain diseases. But it isn't clear whether this is because of the antioxidants, something else in the foods, or other factors. Free

radicals may be formed through natural human physiological processes as well as from the environment. They may be the result of diet, stress, smoking, alcohol, exercise, inflammation, drugs or exposure to sunlight and air pollutants.

While there are many types of free radicals that can be formed, the most common in aerobic (oxygen breathing) organisms are oxygen free radicals, often referred to as Reactive Oxygen Species (ROS), which include superoxides, hydroxyl anions, hydrogen peroxide and singlet oxygen [25]. A free radical is an atom or group of atoms that has an unpaired electron and is therefore unstable and highly reactive. Secondary metabolites are chemicals produced by plants for which no role has yet been found in growth, photosynthesis, reproduction, or other "primary" functions. These chemicals are extremely diverse; many thousands have been identified in several major classes. Each plant family, genus, and species produce a characteristic mix of these chemicals, and they can sometimes be used as taxonomic characters in classifying plants. Humans use some of these compounds as medicines, flavorings, or recreational drugs.

Mollugo nudicaulis belongs to the family Molluginaceae which is prevalent commonly during the rainy season. *Mollugo nudicaulis* is traditionally used to treat whooping cough and jaundice. The ethanolic extract of MN shown antioxidant, antimicrobial, hepatoprotective and anti-diabetic activity [30, 29, 12]. The literature survey revealed that so far, no scientific studies carried out on phytochemical identification and confirmation using LC-MS analysis of *Mollugo nudicaulis* leaves. Hence, in the present study, we focused to evaluate the phytochemical content, anti-oxidant, antimicrobial ability of *Mollugo nudicaulis(MU* and docking its active compounds against SARs COV2 receptor.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Nagesh et al 2011[45], The studied evaluating and comparing wound-healing activity of aqueous extracts of *in vitro*–derived calli and field- grown leaves of *Mollugo nudicaulis* using excision wound model in albino rats. Callus was induced from leaf explant of the species on Murashige and skoog's medium fortified with cytokinins and auxins. Aqueous extract of *in vitro*–derived calli promoted greater wound healing in excision wound models than field-grown leaf extracts. The calli extract–treated animal groups showed increased wound contracting ability and decreased period of epithelialization. An increase in hydroxyproline content was also observed in the calli extract–treated groups. The data of this study indicate that the leaf extract of *in vitro*–derived calli possesses greater wound-healing activity than the leaf extract of field-grown plants.

Rameshkumar et al., 2012[46] studied about the phytochemical tests of *Mollugo nudicaulis* revealed the presence of alkaloid, steroids, flavonoids and reducing sugar in the both aqueous and methanolic extracts. Terpenoids were absent in both aqueous and methanolic extract of *Mollugo nudicaulis*. The total phenolics content of the methanolic and aqueous extract of leaves was 47.01 ± 0.8 and 46.4 ± 0.05 mg/100 g. The total flavonoid content was 41.3 ± 0.04 and 36.2 ± 0.01 mg/100 g respectively. The methanolic and aqueous extract of leaves showed IC_{50} values of DPPH radical scavenging as 48 and 190 µg/ml respectively. The IC_{50} values of ABTS radical scavenging for methanolic of aqueous extracts was 83 and 198.3 µg/ml of plant extract respectively. The total phenolics and flavonoids content and *invitro* antioxidant activity of methanolic extract was higher compared with aqueous extract. The methanolic extract of *Mollugo nudicaulis* used to determine antibacterial activity against bacterial species namely Pseudomonas aeruginosa, Proteus sp, Streptococcus sp, Entrobacter

sp. This investigation suggests that the methanolic extracts of *Mollugo nudicaulis* possess potential antioxidant and antibacterial compounds.

Lotmani, B. et al (2014)[47] , Results shown of preliminary phytochemical screening of leaf, flower and stem of *T. hirsuta* revealed the presence of tannins, alkaloids, steroids, saponins, coumarins, reducteurs compound and anthraquinones. The total phenolics and flavonoids were estimated. The aqueous extracts of the aerial parts of *T. hirsuta* showed potent *in vitro* antioxydant activities using various models *viz,* DPPH scavenging assay, ferric reducing antioxidant power (FRAP) and ABTS radical scavenging activity.

S. Maneemegalai. et al 2018[48], The evaluated of the spectrum of antimicrobial activity of a drug preparation or a plant extracts plays a significant role in therapeutics. The methanolic and ethanolic extracts of the whole plant of *Mollugo cerviana* were tested against common pathogens infecting wound by agar disc diffusion method. Sterile filter paper discs loaded with plant extract at a concentration of (10 mg/ml) tested against the pathogenic strains. Filter paper discs loaded with 5 µg of Tetracycline was used as positive control. Pure solvents serve as negative control. The study has revealed the significant antibacterial activity shown by the methanolic and ethanolic extracts of the whole plant against common pathogens infecting the wounds including *Staphylococcus aureus* and *Pseudomonas aeruginosa* besides other pathogens.

Umamaheswari, K . et al (2017)[49] The investigated the phytochemical constituents, the antimicrobial, antioxidant activity, the functional groups by FT-IR and the presence of bioactive compounds by GC-MS analyses of leaves of Solanum torvum, rhizome of Acorus calamus and whole plant of Mollugo pentaphylla. The preliminary phytochemical analysis of the solvent extracts revealed the presence of alkaloids, flavonoids, steroids, proteins, carbohydrates, phenols, saponins, glycosides, coumarins, quinines. The solvent extracts showed antibacterial activity against

E.faecalis, E.coli S.aureus, B.subtilis. K.pneumonia and C.albicans. The total phenolic content was 0.3430 GAE/g DW whereas, the total flavonoid content varied between 4.928 - 7.751mgQE/g DW. DPPH free radical scavenging activity of the diethyl ether extract of S. torvum recorded the IC50 at a concentration of 7.23µg/ml. In the hydrogen peroxide assay of the M. pentaphylla aqueous extract the IC50 value at a concentration of 3.295µg/ml was recorded. FT-IR studies showed the major functional groups alcohols, alkane, amines, aldehydes, alkyl halide and GC-MS studies showed 20 compounds with phytol being the major constituent.

AIM AND SCOPE

3. AIM AND SCOPE

The study has aimed to reveal the antioxidant and antimicrobial activities of the *M. nudicaulis* through following objectives

- Extraction and optimization of total phenolics and flavonoids
- *Invitro* anti-oxidant activity analysis
- *Invitro* anti-microbial activity analysis
- Polyphenolics screening through LC-MS
- Molecular docking analysis of *Mollugo nudicaulis* compounds against SARs CoV2 receptor.

SCHEME OF STUDY

4. SCHEME OF THE STUDY

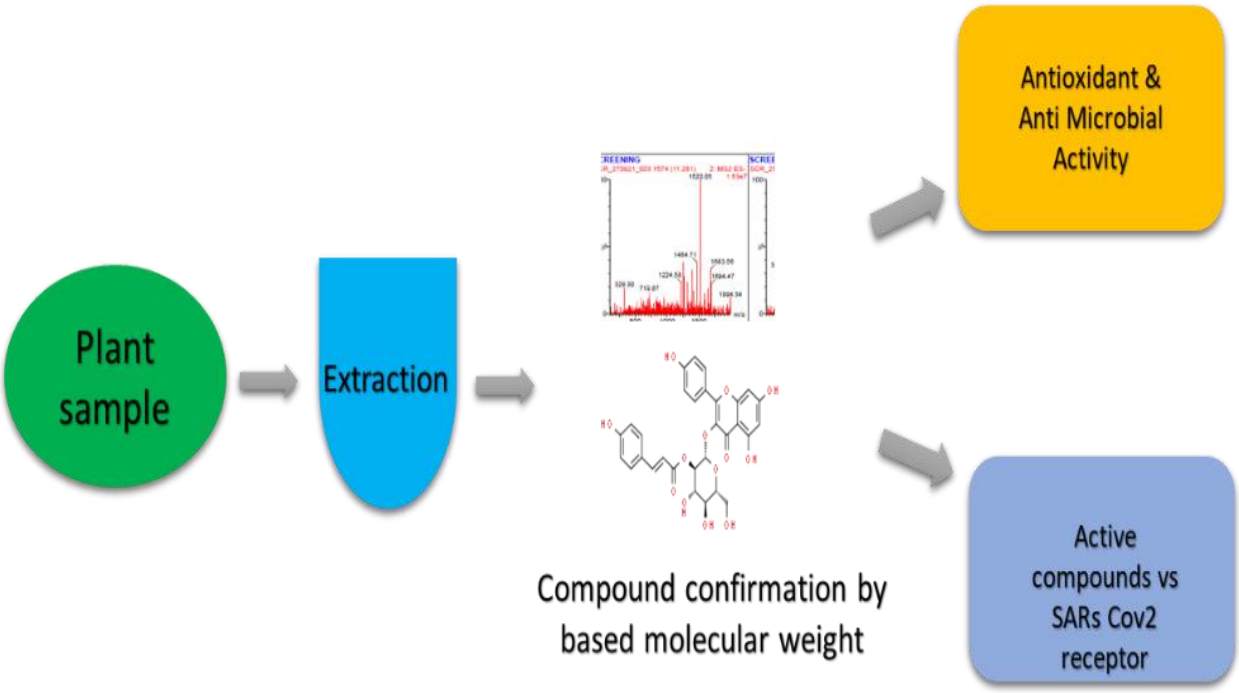


Figure 2: Schematic representation of the study work-flow

EXPERIMENTAL SELECTION

5. EXPERIMENTAL SELECTION

4.1. Determination of total phenolics

Total phenolic content of aqueous and methanolic extracts of *MN* were determined by the modified Folin-Ciocalteu method [15]. 100 µL of Folin-Ciocalteu reagent and 200 µL of Na2CO3 (2% w/v) was added to 100 µL of plant extract solution (1 mg/ml). The resulting mixture was incubated at 45 µL with shaking at 120 rpm for 15 min. The absorbances of the samples were measured at 765 nm using UV-visible spectrophotometer. Results were expressed as milligrams of gallic acid equivalent/g of plant extract. The same procedure was used for making standard curve using gallic acid and concentration range of 0-10 µg/ml was taken. All experiments were carried out in triplicates.

4.2. Estimation of total flavonoids

Total flavonoids content was determined using for Aluminum chloride method [16.] One ml of sample (1 mg/mL) was mixed with 3 mL of methanol, 0.2 ml of 10% aluminium chloride, 0.2 mL of 1 M potassium acetate and 5.6 mL of distilled water and kept at room temperature for 30 min[63]. The absorbance of the reaction mixture was measured at 420 nm using UV-Visible spectrophotometer. The total flavonoid content was determined from calibration curve made by rutin as standard (0-100 µg/mL in methanol). The concentration of total flavonoids was expressed as mg of rutin equivalent/g of plant extract[67].

4.3. Determination of reducing power

The reducing power was determined the following procedure described by [19, 20]. The mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of K3Fe(CN)6 (1%w/v) was added to 1.0 ml of the extract dissolved in distilled water[51]. The resulting mixture was incubated at 50 for 20 min, followed by the addition of 2.5 ml of TCA (10% w/v). The mixture was centrifuged at 5000 rpm for 10 min to collect the upper layer of the solution (2.5 ml), mixed with distilled water (2.5 ml) and 0.5 ml of FeCl3 (0.1%, w/v). The absorbance was measured at 700 nm against blank sample[52].

4.4. 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[62]. The percentage of scavenging inhibition of superoxide radicals was calculated from the equation in Section 2.8.

Superoxide radical Scavenging activity (%) = (Ac − As) (Ac) ×100

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

4.5. Chromatographical analysis

4.5.1. Optimization of RP-HPLC method

The analytical HPLC system employed consists of high-performance liquid chromatography (Waters, USA) coupled with a photodiode array detector (PDA-2998, USA). A C18 reverse phase

column of 4.6×250 mm, 5 μm particle size (SYMMETRY) was used. The mobile phase used was water with 0.1% formic acid as solvent A and 100% ethanol as solvent B[65]. The different isocratic and gradient programs were followed for phenolic compound separation. The optimized gradient program 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[66]. The monitoring wavelength was detected between 210 and 400 nm. The analytical data was evaluated using EMPOWER 2 data processing software from Waters [30]. Fractions were collected for each peak isolated in HPLC.

4.5.2. ESI-MS

UPLC–MS analyses were carried out using an ultra-performance liquid chromatography apparatus equipped with PDA detector (Waters, USA)[69]. Each fraction collected from HPLC was directly injected in ESI-MS, direct infushion method followed in MS. Mass spectroscopic analysis of phenolic compounds in the sample was performed using a SYNAPT mass spectrometer (Waters)[54], equipped with an electrospray ionization source operating in negative ion modes, mass range 100 to 2000 was used in scan method. Phenolic compounds of *MN* leaves were tentatively characterized by comparison with their UV–vis absorption spectra and comparison of MS m/z with reference standards and/or literature reports[56].

4.6. Minimum inhibitory concentration

The antimicrobial activity was carried out using minimum inhibitory concentration method. Samples were diluted in distilled water with 10% of dimethyl sulfoxide DMSO because of solubility of flavonoids[56]. 100 μL of fruit sample solution was added to the sterile 96 well plate containing 100 μL media (Mueller-Hinton) and 100 μL of bacterial species (*E-coli* (ATCC25922), *S. aureus* (CMCC(B)26003) and *B. subtilis (MTTC N0-10110)) used as reference material*[68]. The microbial

suspension was mixed and absorbance were calculated[58]. 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[59].

4.7. Molecular docking:

For insisting 2GZ8-Structure-based drug design and structural biology study of novel nonpeptide inhibitors against SARS-CoV Main Protease, we have performed a molecular docking to give the proof for our concept that quercetin could be a potential drug against novel coronavirus using 1-Click Docking software.

4.7.1. Preparation of protein structure

The three-dimensional crystallographic structure of the native structures of 2GZ8 proteins of coronavirus were retrieved from RCSB Protein Data Bank with a resolution of 1.85Å. The non-essential water molecules, inhibitors, and other complex molecules from protein structure were expelled. The polar hydrogen and Kollaman charges were included in the retrieved protein structure using Discovery Studio 2017 R2.

4.7.2. Preparation of ligand structure

The three-dimensional crystallographic structures of selected LC-ESI-MS/MS derived compounds were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) in .sdf format. The ligand structures were then subjected to an energy minimization process based on the mmff94 force field through which low energy conformer of the ligands was optimized. Once bond orders were fixed, the energy minimized ligands were subjected to docking analysis.

4.7.3. Molecular docking analysis

Molecular docking was performed with 2GZ8 proteins of coronavirus receptor, using PyRx 0.8 (AutoDock inbuild Vina) according to Dallakyan et al. (2015). Initially, the energy minimized three-dimensional structures of ligands and SARs Cov2 were converted to PDBQT format[67]. The molecular docking was performed based on a rigid docking approach (Lamarckian Genetic Algorithm) where the grid-box parameters, X=44.6658; Y=16.5763; Z=9.0326 and dimensions X=43.76; Y=35.14; Z=37.47 were covered amino acids in the active sites. The binding mode and binding affinities were calculated using the 1-Click Docking software.

4.7.4. Visualization and Interaction studies

The visualization of SARs Cov2-polyphenols interaction including binding sites identification, hydrogen bond interactions with amino acids, Pi-Alkyl, Pi-Pi, Pi-Sigma interactions, binding energy (Kcal/mol) calculation, distance, and angles identification were performed using Discovery Studio 2017 R2 software.

RESULT AND DISCUSSION

6. RESULTS & DISCUSSION

5.1. Total Polyphenolics and flavonoids content

The relative abundance of total phenolics (TPC) and flavonoids content (TFC) of any food or medicine defines its therapeutic potential [24]. The study has determined of TPC and TFC in 90% ethanolic extract of *M. nudicaulis* through spectrophotometrically and calculated both content respectively as Gallic acid and Rutin equivalents using linear regression from standard curves. The total phenolics content of *M. nudicaulis* ethanolic extract was found to present slightly higher than the flavonoid content which is presented in the table 1 below (50 ± 0.85 mg GAE/g vs 48.52 mg/kg ± 0.49 mg GAE/g of the dry weight of the extracts).

Extract	TPC (mg of GAE/g)	TFC (mg of RE/g)
HPAQ	50.00 ± 0.85	48.52 ± 0.49

Table 1: Estimation of total phenolics and flavonoids content of *M. nudicaulis* extract. The results are expressed as mean ± SEM (n = 3).

5.2. Reducing power Results

The reducing properties of antioxidants are generally associated with the presence of reductones, such as ascorbic acid and other secondary metabolites. Such reductones exert antioxidant action by breaking the free radical chain by donating hydrogen atoms; Reductones have also been reported to react with certain precursors of peroxide, thus preventing peroxide formation [24]. In the presence of antioxidants in the sample, would result in the reduction of Fe 3+

to Fe 2+ by donating an electron. The amount of Fe 2+ complex can then be monitored by measuring the absorbance at 700 nm. In the present study, the reducing power of the methanolic extract of Mollugo nudicaulis was found to be steadily increased in direct proportion to the increasing concentration of the extract (Figure 3). The reducing power of ethanolic extract of *Mollugo nudicaulis* and BHT at 0.5 mg/mL concentration was total reducing power absorbance shows 1.1 and 2.21 mg/ml respectively.

Concentration mg/ml	0	0.1	0.2	0.3	0.4	0.5
Absorbance Sample	0	0.11	0.26	0.38	0.81	1.1
Absorbance BHT	0	0.32	0.66	0.97	1.65	2.21

Table 2: Effect of *M. nudicaulis* extracts on Ferric reducing power.

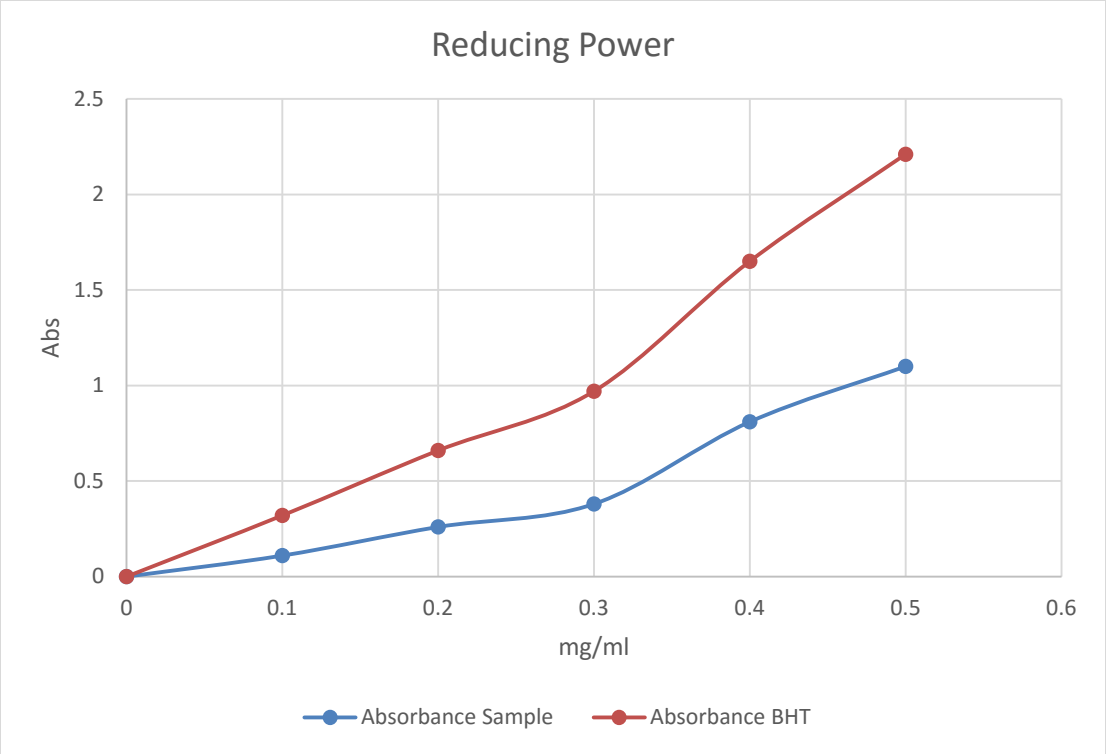


Figure 3: Effect of *M. nudicaulis* extract on Ferric reducing power. The results are expressed as mean ± SEM (n = 3).

5.3. Superoxide anion scavenging activity (SRSA)

. The contents of total phenolics and total flavonoids and the IC50 values of eleven plum cultivars are listed in Table 2. BY 69-339 variety exhibited the lowest IC50 (1.71 g fresh sample in 100 mL of 50% MeOH) among the tested plums, which means powerful antioxidant activity in scavenging superoxide radicals, followed by French Damson, Cacaks Best, Beltsville Elite B70197, Empress, Castleton, Stanley, NY 6, NY 101, Mirabellier, NY 9. BY 69-339 had 4.8 times higher SRSA. In this present study results of superoxide anion scavenging activity IC_{50} value of extract and BHT shows 152 µg/ml and 120 µg/ml respectively.

The IC50 of individual phenolic compounds using SRSA assay. According to the results, the relative IC50 of cyanidin was about 14 times higher than that of chlorogenic acid, and about 3.1 times higher than quercetin 3-rutinoside. In a previous study [31], quercetin was reported to possess higher SRSA than chlorogenic acid, quercetin glucoside, and quercetin galactoside. Sichel et al. [33] reported that the effectiveness of flavonoids in superoxide radical scavenging, in alkaline medium by ESR spectrometry, was in the order of quercetin > quercitrin > cyanidin > quercetin 3-rutinoside > peonidin. The different aspect of the result with present study may be attributed to different methods of assessment, varying substrate conditions, and differential concentrations of active components. Although there is a wealth of data on the importance of SRSA, the correlation between SRSA and chemical structure is far from clear. The SRSA analyzed in this study is carried out under the similar condition with our body substrate system.

SRSA of Plums

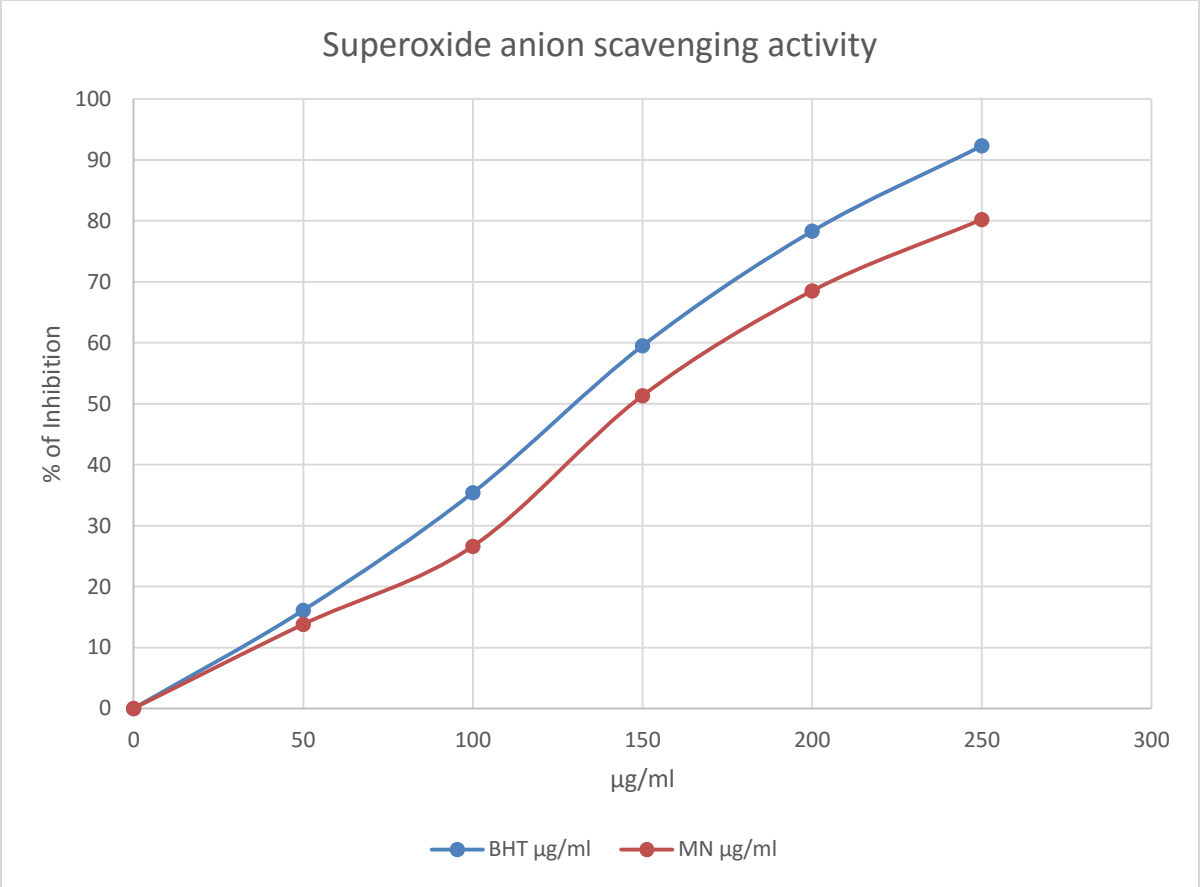


Figure 4: Effect of *M. nudicaulis* extract on Superoxide scavenging capacity. The results are expressed as mean ± SEM (n = 3).

Concentration µg/ml	0	50	100	150	200	250
BHT µg/ml	0	16.1	35.4	59.5	78.3	92.3
MN µg/ml	0	13.8	26.6	51.3	68.5	80.2

Table 3: Percent of inhibition by *M. nudicaulis* extract on Superoxide scavenge. The results are expressed as mean ± SEM (n = 3).

5.4. Chromatographical analysis

The phenolics and their derivatives present in *M. nudicaulis* were revealed using LC-ESI-MS analysis in both positive and negative ionization modes. The identity of detected polyphenolics was obtained by matching their molecular mass of parental ion and MS^2 fragmentation pattern reported in earlier literature. Totally, 13 different compounds were detected in (Table 2) that have been reported for the management of various diseases including diabetes, cancer etc.

The total ion chromatograms showed the abundance of compounds on both positive and negative modes in the above figure. Compounds such as Rutin, Kaempferol-3-O-Glucuronide, and Physcion 8-O-glucoside are identified in *M. nudicaulis* from the present study. These are familiar flavonoids and their pharmaceutical values have been investigated for various diseases. Chemical structures of the identified compounds from *M. nudicaulis* were downloaded from PubChem-NCBI and depicted in the figure (). The details of retention time and mass details of those compounds were presented in the table 4.

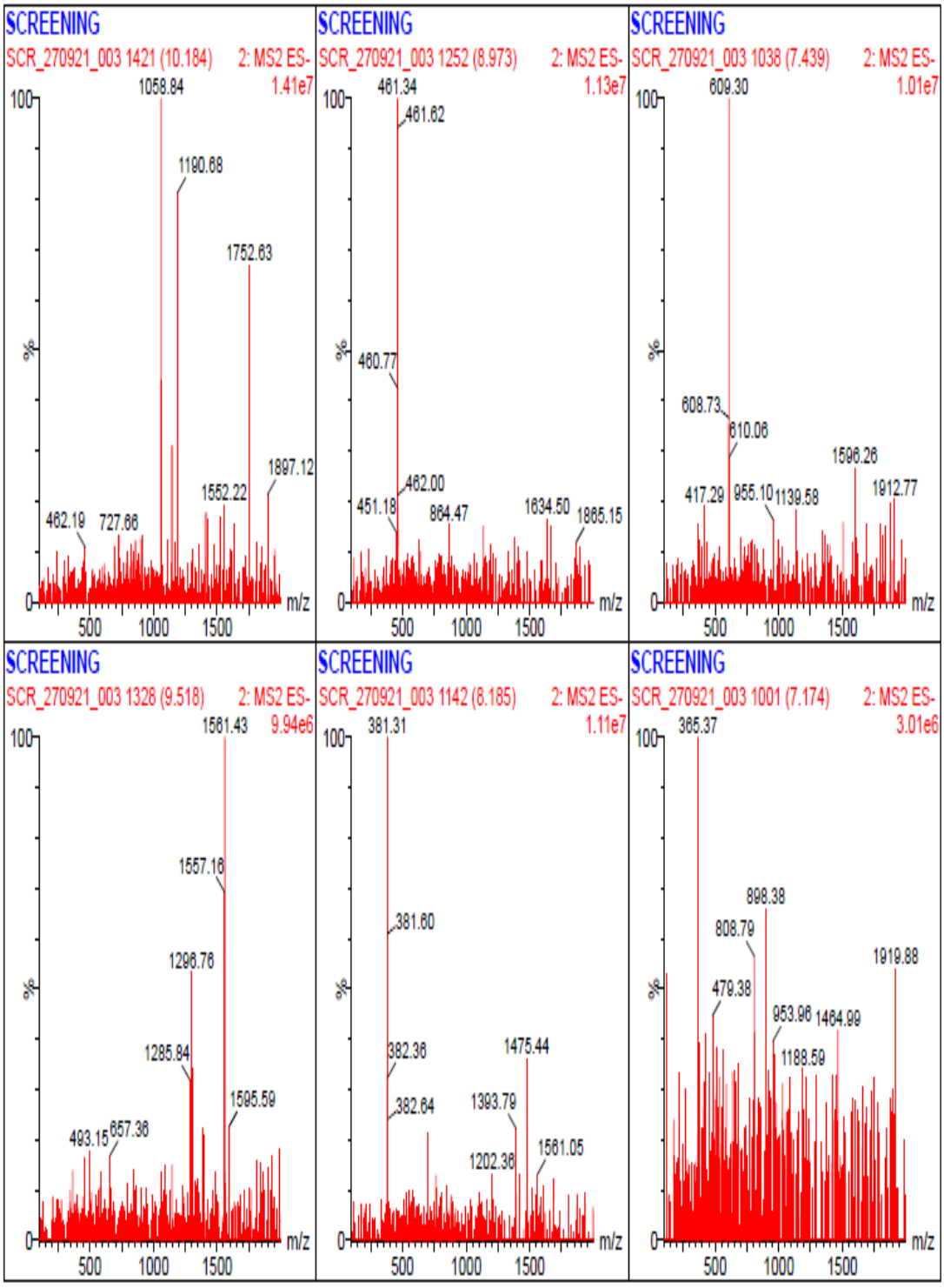


Figure 5: Total ion chromatogram of ethanolic extract of *M. nudicaulis* in positive and negative ESI modes

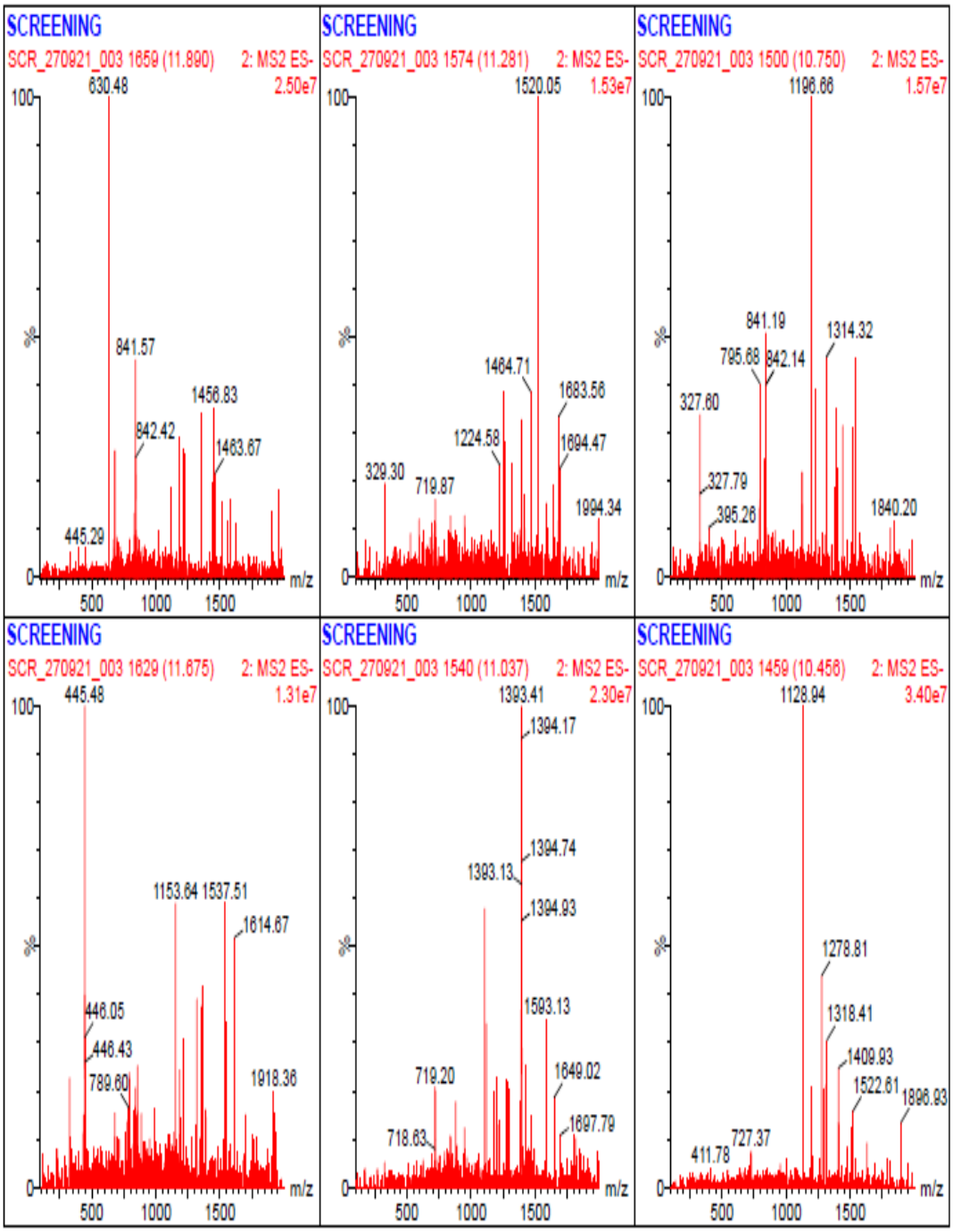


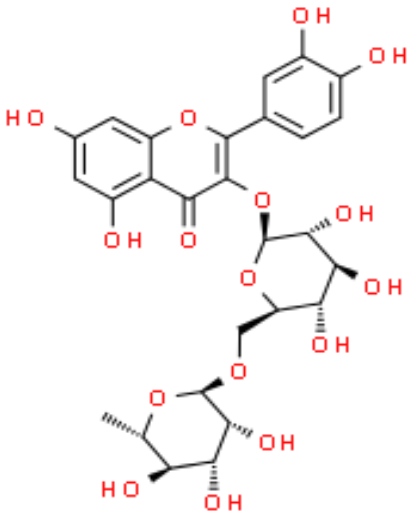
Figure 6: Total ion chromatogram of ethanolic extract of *M. nudicaulis* in positive and negative ESI modes

Table 4: Identification of polyphenols in the active extracts of *M. nudicaulis* through LC-ESI-MS analysis

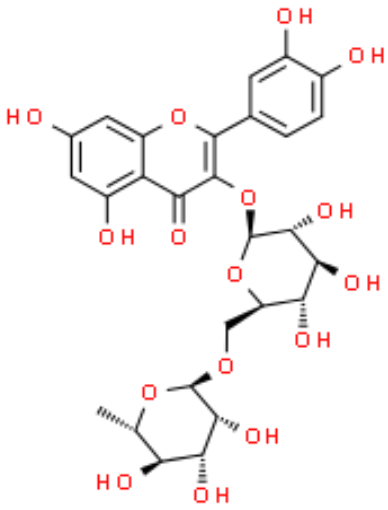
S.No	RT	Mode	Ion (m/z)	Compounds	Reference
		[M-H]			
1	7.174	_	365.37	Unknown	
2	8.18	_	381.31	Unknown	
3	7.439	_	609.3	Rutin	Sayed et al, 2020
4	8.973	_	461.34	Kaempferol-3-O-Glucuronide	Sayed et al, 2020
5	9.518	_	1561	Unknown	
6	10.18	_	1058.8	Unknown	
7	11.48	_	630	Unknown	
8	10.45	_	1128.9	Unknown	
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



Physcion 8-O-glucoside

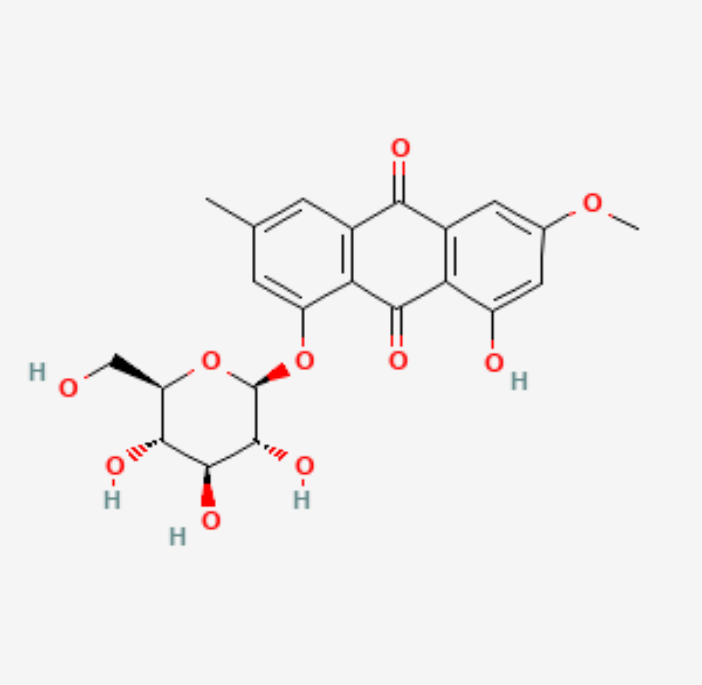


Figure 7: Structure of the compounds identified from *M. nudicaulis* through LC-ESI-MS

Rutin was detected in *M. nudicaulis* extract through LC-ESI-MS analysis at the mass of 609.03. It is a well-known flavonoid reported for hepatoprotective, antioxidant, anti-inflammatory activities and anticancer properties. It is applied in the therapeutic composition for the treatment of diabetic and aging complications also [58]. It inhibits angiogenesis and decreases the progress of many different types of cancers [59]. **Physcion 8-O-glucoside** was detected in *M. nudicaulis* extract through LC-ESI-MS analysis at the mass of 282.85. It is one of the most prevalent alkaloids, widely distributed in vegetables, fruits and herbs. Recent studies have attributed a preventive potential of luteolin for various pharmacological benefits including anti-oxidant, anti-tumor, anti-inflammatory, and anti-apoptotic activities [60,61]. It is also found

effectively protect multitude of CVD, including coronary artery disease, heart failure, and atherosclerosis [62].

Moreover, **Kaempferol 3-O-glucuronide**, a well characterized natural flavonol was detected in *M. nudicaulis* extract through LC-ESI-MS analysis at the mass of 461.34. It is present in most of plant-based foods, including broccoli, kale, cabbage, leek, tomato, beans, grapes, strawberries, apples, and tea [63]. Recent studies have shown antiproliferative and proapoptotic activities of Kaempferol derivatives against various types of cancers [64]. It has been reported that Kaempferol derivatives have potential to effectively modulate a variety of signalling pathways related to inflammation and oxidative stress [65].

5.5. Minimum inhibitory concentration

The antimicrobial activity of ethanolic extract of *M. nudicaulis* was determined with known quantities ranging 10µg/ml to 200µg/ml against *E. coli, S. aureus* and *B. subtilis cultures*. The minimal inhibitory concentration of the plant extract was found to be 43.2 ± 3.0, 22 ± 2.0 and 21.0 ± 4.0 µg/ml respectively against the tested microorganisms; *E. coli, S. aureus* and *B. subtilis*. The extract has proactively inhibited the activity of *B. subtilis* at very minimal concentration which is comparatively better than with the inhibition of *S. aureus and E. coli activities (*21.0 ± 4.0 µg/ml vs 22 ± 2.0 and 21.0 ± 4.0 µg/ml vs 43.2 ± 3.0 µg/ml*)*

Plant based antibiotic drugs have enormous therapeutic potential and have been proven effective in the treatment of infectious diseases with less or no side effects which are often associated with synthetic antibiotics [66.] Previous reports suggest that phenolic and flavonoid compounds (gallic acid, caffeic acid, p-coumaric acid, quercetin, rutin and catechin) in plants possess strong antioxidant activity that might contribute to antimicrobial potential [67]. The terpene alcohols

damage the cell membranes of E. coli, S. aureus, and Listeria monocytogenes, resulting in leakage of potassium ions from cells, which cause death of the organism. Recent reports suggest that the sesquiterpene alcohol like farnesol has been confirmed to reduce the growth of S. aureus and Streptococcus mutans [68, 69.] From these results, we have identified the major bioactive compounds in *M. nudicaulis* which may have many offered antimicrobial activities.

5.6. Molecular docking:

For insisting 2GZ8-Structure-based drug design and structural biology study of novel nonpeptide inhibitors against SARS-CoV2 Main Protease, we have performed a molecular docking to give the proof for our concept that quercetin could be a potential drug against novel coronavirus. The active sites of the selected proteins were identified using 1-click docking. The 3D structures of the proteins and the ligand were loaded into molecular docking software. After identifying the active sites, the docking grid was set accordingly and the main docking process was performed.

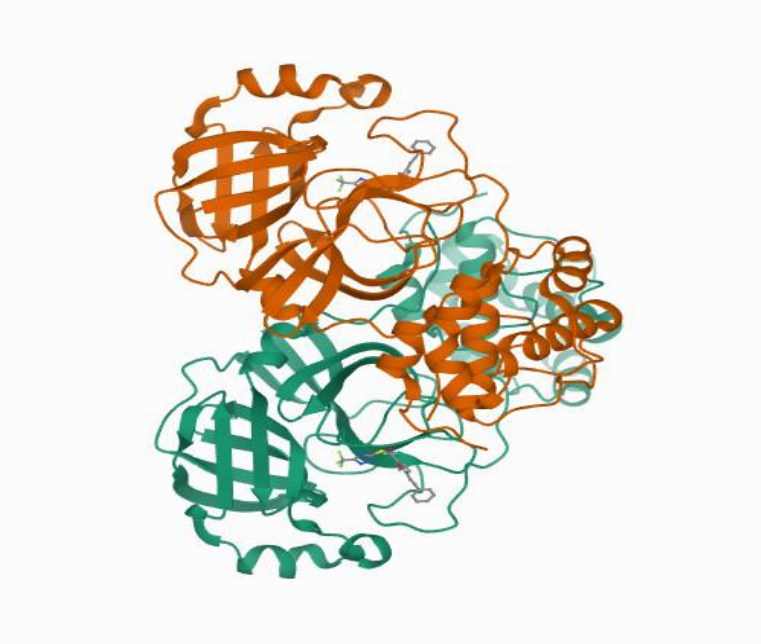


Figure 8: 2GZ8 Protease Structure (Reference: RCSB.Org)

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

Table 5: Prediction of docking score (binding energy) for the compounds identified from *M. nudicaulis*

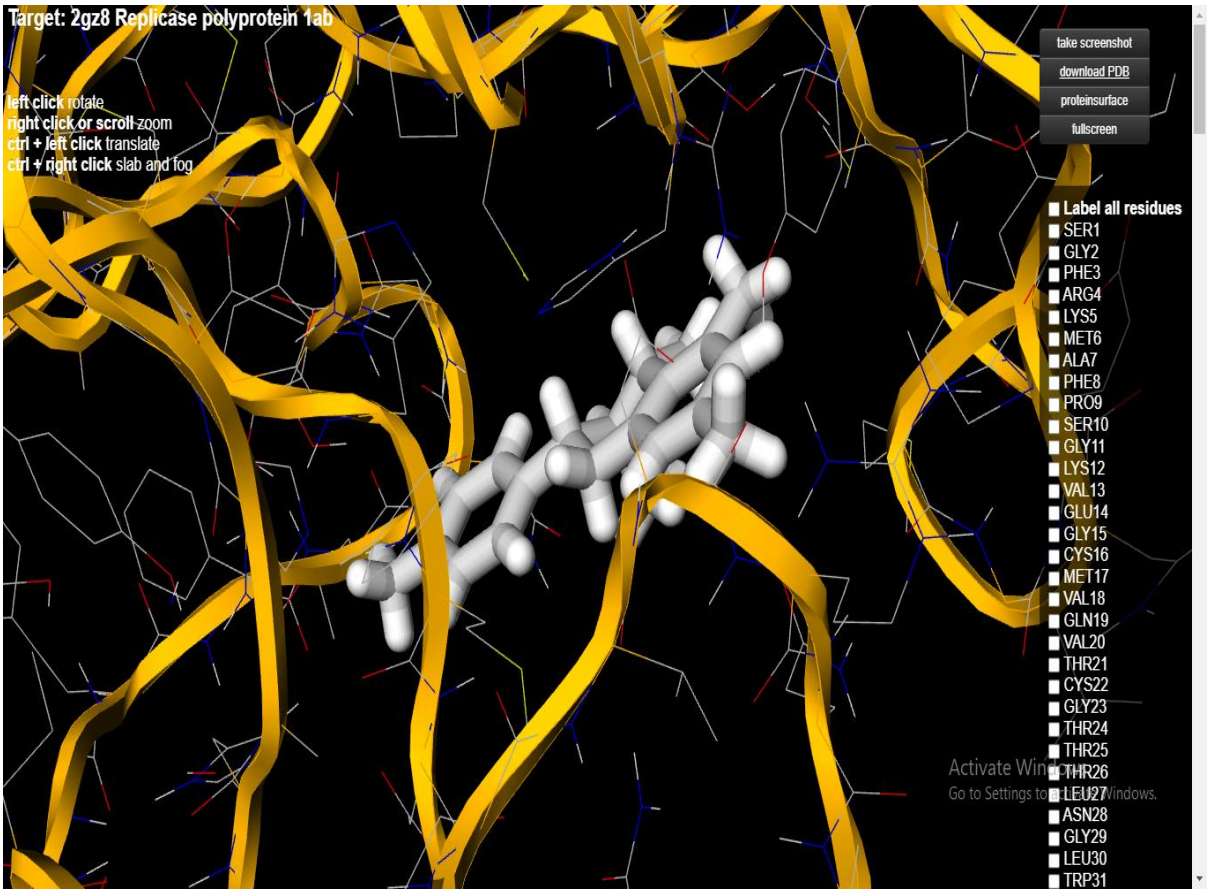


Figure 9: Molecular docking of Compound 1–SARs CoV2 receptor complex

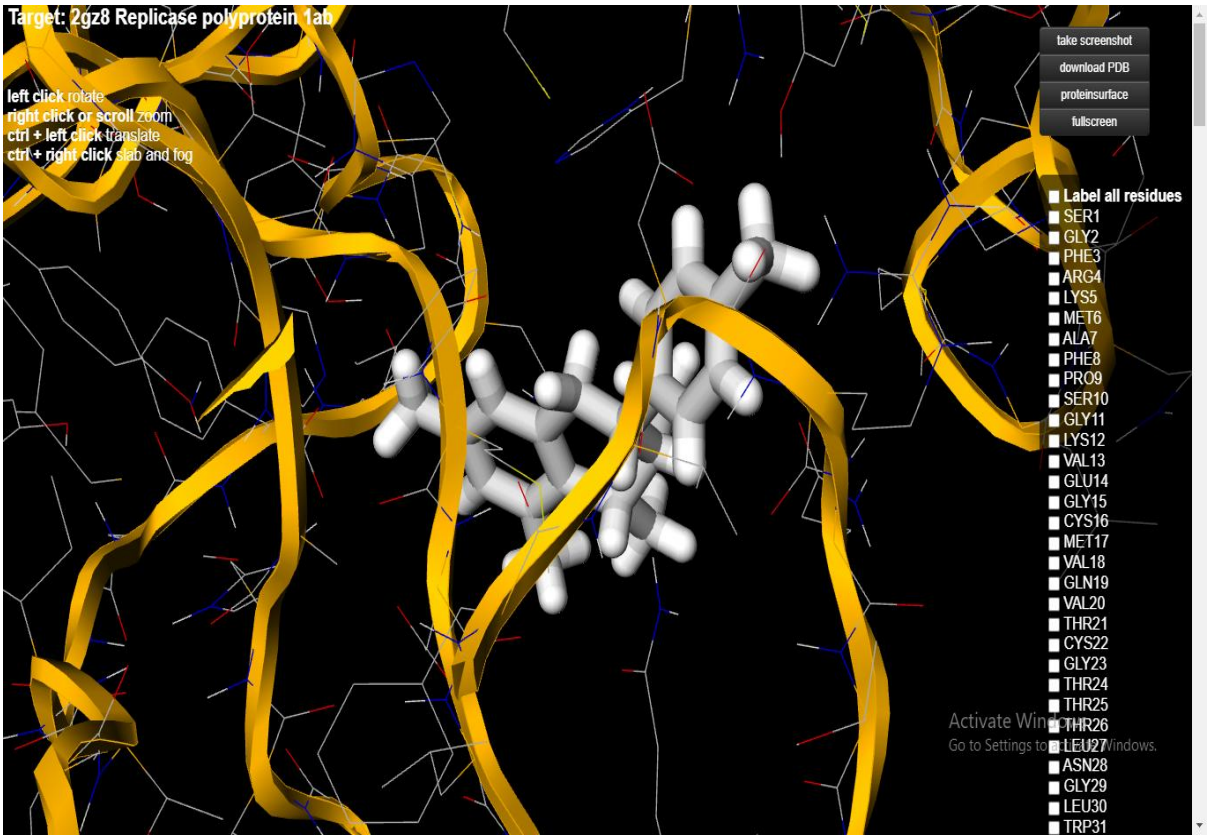


Figure 10: Molecular docking of Compound 2–SARs CoV2 receptor complex

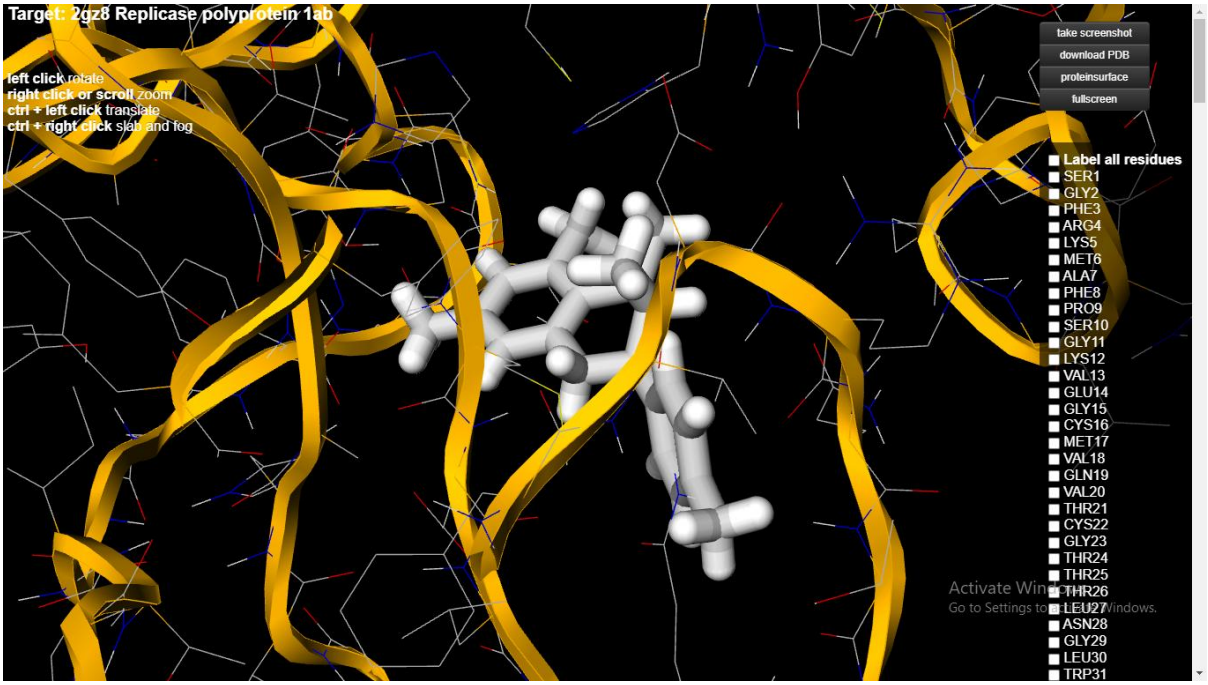


Figure 11: Molecular docking of Compound 3–SARs CoV2 receptor complex

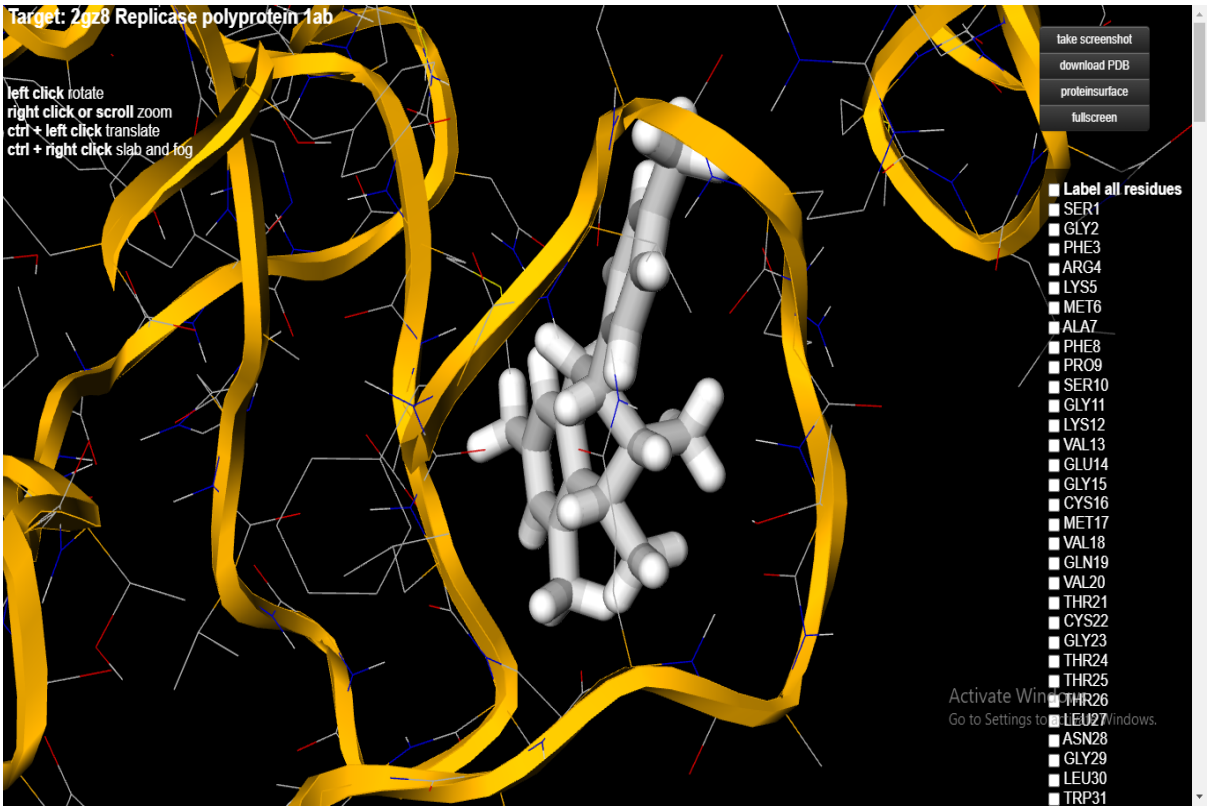


Figure 12: Molecular docking of Compound 4–SARs CoV2 receptor complex

The molecular docking of the energy minimized ligands with primary target, SARs CoV2 receptor was performed using AutoDock inbuilt PyRx software. Active site residues of SARs CoV2 receptor protein predicted against *M. nudicaulis* compounds were found almost similar to pre-existing ligands. This was confirmed as the selected mmff94 force field optimization was found fit to all the selected ligands. Followed by the binding energy of ligands (*M. nudicaulis* compounds) with SARs CoV2 was studied and results showed that Kaempferol 3-O-glucuronide contain minimized binding energy with SARs CoV2 receptor protein (Table 5). Among these, three potential ligands with less binding energy and good hydrogen bond interaction were chosen (Table 5), and their protein-ligand complex was visualized.

The grid-box based docking results visualized under Discovery studio showed the binding pose of ligand-protein complex (Figure 9). Among the chosen ligands, hydroxyl was observed to be the best interacting ligand with SARs CoV2 receptor target protein complex with exhibiting four H-bond, two C-H bond interactions, an electrostatic interaction, and one Pi-Alkyl interaction with minimized binding energy (Figure 10). Followed by amino acids active sides showed better interaction with SARs CoV2 receptor target protein through three covalent bond interactions, an electrostatic bond, and two hydrophobic interactions with minimized binding affinity as shown in Figure 11. Furthermore, kaempeferol showed three hydrogen bonds, one C-H bond, an electrostatic interaction, and two hydrophobic interactions with SARs CoV2 receptor target protein (Figure 12).

The Molecular Docking analysis evidenced those amino acids such as essential amino acids were played a crucial role in the ligand interactions and shown in Table 1 and Figure 10. The hydrogen bond pattern has strongly existed for the affinity between the protein and ligand. The Molecular Docking analysis was initiated with re-docking of *M. nudicaulis* compounds against SARs CoV2 receptor target protein showing the range of binding energy between -0.800 to -0.610 kcal/mol. Here, the ligands were analyzed according to the hydrogen bond interaction and they found interacted high proportionally with amino acids such as active residues of the SARs CoV2 receptor protein.

The purpose of performing molecular docking is to find the possible interactions of a protein-ligand complex. Hydrogen bond interaction is one of the key parameters that influence strongly the pharmacokinetic properties of ligands [70]. From the study, the best interacting compounds of ***M. nudicaulis*** were screened using Glide-XP (Table 1). Upon manual clustering,

the outcome of Glide-XP has identified the best compounds based on their docking scores. The screened compounds have shown the very good binding energy.

In addition to hydrogen bridging, the hydrophobic interaction is also considered to play a vital role by enhancing the stability of molecular interactions between protein-ligand complex [70]. Here, the hydrophobic amino acids; glycine, arginine and other are surrounded nearby the interacting area is believed to provide strong stability to protein-ligand complexes during molecular docking process. Based on the obtained docking results, ligands such as kaempeferol glucosides were screened as the top polyphenolic candidates of ***M. nudicaulis*** to be capable of interacting and inhibiting SARs CoV2 receptor protein in the molecular docking process.

SUMMARY AND CONCLUSION

7. SUMMARY AND CONCLUSION

Summarily, 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 butylated hydroxytoluene (BHT) employing superoxide anion scavenging activity and total 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 *Mollugo nudicaulis* leaves. Hence, in the present study, we focused to evaluate the phytochemical content, docking and antimicrobial ability of *Mollugo nudicaulis*.

In this study, aqueous and methanolic extracts of MN were taken for analysis, methanolic extract exhibited higher antioxidant activities in all the three antioxidant assays performed (ABTS, DPPH and reducing power). The antioxidant activities of methanolic extracts from the leaves and stems of were assessed in various herbal plants in an effort to compare and validate the medicinal potential by quantifying phenolic, flavanoid contents and by antioxidant assays such as DPPH, ABTS and ferric reducing power assays. Our results indicate that presence of significant quantity of total phenolis and flavonoids and antioxidant activity in *MN* leaves. This makes us to interpret that *MN* leaves possess promising health beneficial phytochemicals.

It is widely accepted that phenolic and flavonoids compounds may significantly contribute to overall antioxidant activities and also to antimicrobial activity. Ok-Hwan Lee and Boo-Yong Lee have purified the phenolic compounds from olive leaves and tested them for antioxidant and antimicrobial activities. Methanolic extract of *MN* leaves showed antibacterial activity against all the four bacterial species tested such as Pseudomonas aeruginosa, Proteus sp, Streptococcus sp and Entrobacter sp. Similar studies were carried out in Merremia emarginata leaves extracts against S. aureus, Staphylococcus epidermidis, E. coli, and P. aeruginosa to test the antibacterial activity. The abovesaid biological properties were significantly attributed to the presence of bioactive compounds of the *M. nudicaulis* which have been revelaed through LC-MS/MS analysis. Moreover, their activities have been further proved through in-silico molecular docking studies in which major bioactive compounds such as Rutin, Physcion 8-O-glucoside, and Kaempferol 3-O-glucuronide showed effective inhibitory interaction with SARs CoV2 receptor. This altogether suggests that *M. nudicaulis* can be a potential source of antioxidants and antimicrobial compounds to combat pandemic viruses.

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 possibilities was confirmed using molecular docking study against Kaempferol. results conclude plant extract were act as good anti-oxidant and anti-microbial agent. Based on our findings we conclude that Mollugo nudicaulis have significant amount of total phenolics and flavonoids. Methanolic extract of Mollugo nudicaulis has shown higher in invitro antioxidant and antimicrobial activity. Further work will be carried out to find biologically active compounds purification and structural elucidation through NMR studies.

BIBLIOGRAPHY

8. BIBLIOGRAPHY

1. K. S. Nagesh & C. Shanthamma (2011) Wound-healing Activity of *Mollugo nudicaulis* in Wistar Albino Rats: Field-grown Leaf Versus *In Vitro*–derived Leaf Calli Extracts, Journal of Herbs, Spices & Medicinal Plants, 17:3, 275-284,.

2. A. Rameshkumar, T. Sivasudha,In vitro Antioxidant and Antibacterial Activity of Aqueous and Methanolic Extract of Mollugo nudicaulis Lam. Leaves,Asian Pacific Journal of Tropical Biomedicine, , Supplement,2012, S895-S900.

3. Amari NO, Bouzouina M, Berkani A, Lotmani B. Phytochemical screening and antioxidant capacity of the aerial parts of *Thymelaea hirsuta* L. *Asian Pac J Trop Dis*. 2014;4(2):104-109.

4. Mothana R A A and Lindequist U. Antimicrobial activity of some medicinal plants of the island Soqotra. J. of Ethnophar 2005; 96: 177-181. [5] Bajpai M, Pande A, Tewari SK and Prakash D. Phenolic contents and antioxidant activity of some food and medicinal plants. Intl J of Food Sci and Nut 2005; 56(4): 287-291.

5. P. Padmapriya, and S. Maneemegalai. "Evaluation of Antimicrobial Activity of the Whole Plant Extract of Mollugo Cerviana (L.)Ser Against Human Pathogens". *International Journal of Pharmaceutics and Drug Analysis*, , Feb. 2018, pp. 127-32, .

6. Mohammad, R. S., Omid, A., Somayeh, S. H., Nejat, K. H., Alireza, P. R., 2018. Antiglycation and antioxidant activity of four Iranian medical plant extracts. Journal of Pharmacopuncture. 21 (2), 82–89.

7. Kaul TN, Middleton E Jr, Ogra PL (1985) Antiviral effect of flavonoids on human viruses. J Med Virol 15(1):71–79.

8. Vrijsen R, Everaert L, Boeyé A (1988) Antiviral activity of flavones and potentiation by ascorbate. J Gen Virol 69(7):1749–1751.

9. Iwashina T (2015) Contribution to flower colors of flavonoids including anthocyanins: a review. Nat Prod Commun 10(3):529–544.

10. Panche AN, Diwan AD, Chandra SR (2016) Flavonoids: an overview. J Nutr Sci 5:e47.

11. Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids:an overview. Sci World J 2013:e162750.

12. David AVA, Arulmoli R, Parasuraman S (2016) Overviews of biological importance of quercetin: a bioactive flavonoid. Pharmacogn Rev 10(20):84–89.

13. Mahmoud MF, Hassan NA, El Bassossy HM, Fahmy A (2013) Quercetin protects against diabetes-induced exaggerated vasoconstriction in rats: effect on low grade inflammation. PLoS One 8(5):e63784.

14. Vafadar A, Shabaninejad Z, Movahedpour A, Fallahi F, Taghavipour M, Ghasemi Y (2020) Quercetin and cancer: new insights into its therapeutic effects on ovarian cancer cells. Cell Biosci 10(1):32.

15. Haleagrahara N, Miranda-Hernandez S, Alim MA, Hayes L, Bird G, Ketheesan N (2017) Therapeutic effect of quercetin in collagen-induced arthritis. Biomed Pharmacother 90:38–46.

16. Jaisinghani RN (2017) Antibacterial properties of quercetin. Microbiol Res 8(1):6877.

17. Sabogal-Guáqueta AM, Munoz-Manco JI, Ramírez-Pineda JR, Lamprea-Rodriguez M, Osorio E, Cardona-Gómez GP (2015) The flavonoid quercetin ameliorates Alzheimer's disease pathology and protects cognitive and emotional function in aged triple transgenic Alzheimer's disease model mice. Neuropharmacology 93:134–145.

18. Sriraksa N, Wattanathorn J, Muchimapura S, Tiamkao S, Brown K,Chaisiwamongkol K (2012) Cognitive-enhancing effect of quercetin in a rat model of Parkinson's disease induced by 6-hydroxydopamine. Evid Based Complement Altern Med 2012:823206.

19. Shoskes DA, Nickel JC (2011) Quercetin for chronic prostatitis/chronicpelvic pain syndrome. Urol Clin 38(3):279–284.

20. Ferreres F, Taveira M, Pereira M, Valentao P, Andrade PB (2010) Tomato (Lycopersicon esculentum) seeds: new flavonols and cytotoxic effect. J Agric Food Chem 58(5):2854–2861.

21. Zhang Y, Li Y, Cao C, Cao J, Chen W, Zhang Y et al (2010) Dietary flavonol and flavone intakes and their major food sources in Chinese adults. Nutr Cancer 62(8):1120–1127.

22. Wang D, Sun-Waterhouse D, Li F, Xin L, Li D (2019) MicroRNAs as molecular targets of quercetin and its derivatives underlying their biological effects: a preclinical strategy. Crit Rev Food Sci Nutr 59(14):2189–2201.

23. Xu D, Hu MJ, Wang YQ, Cui YL (2019) Antioxidant activities of quercetin and its complexes for medicinal application. Molecules 24(6):e1123.

24. Chae HS, Xu R, Won JY, Chin YW, Yim H (2019) Molecular targets of genistein and its related flavonoids to exert anticancer effects. Int J Mol Sci 20(10):e2420.

25. Jan AT, Kamli MR, Murtaza I, Singh JB, Ali A, Haq QMR (2010) Dietary flavonoid quercetin and associated health benefits - an overview. Food Rev Int 26(3):302–317.

26. Nijveldt RJ, van Nood E, van Hoorn DEC, Boelens PG, van Norren K, van Leeuwen PAM (2001) Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr 74(4):418–425

27. Veckenstedt A, Pusztai R (1981) Mechanism of antiviral action of quercetin against cardiovirus infection in mice. Antiviral Res 1(4):249–261.

28. Ganesan S, Faris AN, Comstock AT, Wang Q, Nanua S, Hershenson MB et al(2012) Quercetin inhibits rhinovirus replication in vitro and

29. Ono K, Nakane H, Fukushima M, Chermann JC, Barré-Sinoussi F (1990) Differential inhibitory effects of various flavonoids on the activities of reverse transcriptase and cellular DNA and RNA polymerase. Eur J Biochem 190(3):469–476.

30. Ohnishi E, Bannai H (1993) Quercetin potentiates TNF-induced antiviral activity. Antiviral Res 22(4):327–331.

31. Wu W, Li R, Li X, He J, Jiang S, Liu S, Yang J (2016) Quercetin as an antiviral agent inhibits influenza A virus (IAV) entry. Viruses 8(1):6.

32. Lee S, Lee HH, Shin YS, Kang H, Cho H (2017) The anti-HSV-1 effect of quercetin is dependent on the suppression of TLR-3 in Raw 264.7 cells. Arch Pharm Res 40(5):623–630.

33. Gravina HD, Tafuri NF, Júnior AS, Fietto JLR, Oliveira TT, Diaz MAN et al (2011) In vitro assessment of the antiviral potential of trans-cinnamic acid, quercetin and morin against equid herpesvirus 1. Res Vet Sci 91(3):e158–e162.

34. Johari J, Kianmehr A, Mustafa MR, Abubakar S, Zandi K (2012) Antiviral activity of baicalein and quercetin against the Japanese encephalitis virus. Int J Mol Sci 13(12):16785–16795.

35. Chiow KH, Phoon MC, Putti T, Tan BK, Chow VT (2016) Evaluation of antiviral activities of Houttuynia cordata Thunb. extract, quercetin, quercetrin and cinanserin on murine coronavirus and dengue virus infection. Asian Pacific J Tropic Med 9(1):1–7.

36. Thapa M, Kim Y, Desper J, Chang KO, Hua DH (2012) Synthesis and antiviral activity of substituted quercetins. Bioorg Med Chem Lett 22(1):353–356.

37. dos Santos AE, Kuster RM, Yamamoto KA, Salles TS, Campos R, de Meneses MD et al (2014) Quercetin and quercetin 3-O-glycosides from Bauhinia longifolia (Bong.) Steud. show anti-Mayaro virus activity. Parasit Vectors 7(1): 130

38. Fan D, Zhou X, Zhao C, Chen H, Zhao Y, Gong X (2011) Anti-inflammatory, antiviral and quantitative study of quercetin-3-O-β-D-glucuronide in Polygonum perfoliatum L. Fitoterapia 82(6):805–810.

39. Qiu X, Kroeker A, He S, Kozak R, Audet J, Mbikay M et al (2016) Prophylactic efficacy of quercetin 3-β-O-d-Glucoside against Ebola virus infection. Antimicrob Agents Chemother 60(9):5182–5188.

40. Wong G, He S, Siragam V, Bi Y, Mbikay M, Chretien M et al (2017) Antiviral activity of quercetin-3-β-O-D-glucoside against Zikavirus infection. Virol Sin 32(6):545–547.

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41. Choi HJ, Kim JH, Lee CH, Ahn YJ, Song JH, Baek SH et al (2009) Antiviral activity of quercetin 7-rhamnoside against porcine epidemic diarrhea virus. Antiviral Res 81(1):77–81.

42. Choi HJ, Song JH, Park KS, Kwon DH (2009) Inhibitory effects of quercetin 3-rhamnoside on influenza A virus replication. Eur J Pharm Sci 37(3-4):329–333.

43. Nguyen TTH, Woo HJ, Kang HK, Kim YM, Kim DW, Ahn SA et al (2012) Flavonoid-mediated inhibition of SARS coronavirus 3C-like protease expressed in Pichia pastoris. Biotechnol Lett 34(5):831–838.

44. Yao C, Xi C, Hu K, Gao W, Cai X, Qin J et al (2018) Inhibition of enterovirus 71 replication and viral 3C protease by quercetin. Virol J 15(1):116

45. Senthilvel P, Lavanya P, Kumar KM, Swetha R, Anitha P, Bag S et al (2013) Flavonoid from Carica papaya inhibits NS2B-NS3 protease and preventsDengue 2 viral assembly. Bioinformation 9(18):889–895

46. Chen L, Li J, Luo C, Liu H, Xu W, Chen G et al (2006) Binding interaction of quercetin-3-β-galactoside and its synthetic derivatives with SARS-CoV 3CLpro: structure-activity relationship studies reveal salient pharmacophore features. Bioorg Med Chem 14(24):8295–8306.

Khaerunnisa S, Kurniawan H, Awaluddin R, Suhartati S, Soetjipto S (2020) Potential inhibitor of COVID-19 Main Protease (Mpro) from several medicinal plant compounds by molecular docking study. Preprints

48. Sampangi-Ramaiah MH, Vishwakarma R, Uma Shaanker R (2020) Molecular docking analysis of selected natural products from plants for inhibition of SARS-CoV-2 main protease. Curr Sci 118:1087–1092.

49. Rameshkumar et al., 2012, investigated *in vitro* antioxidant and antibacterial activity of aqueous and methanolic extract of *Mollugo nudicaulis* Lam. Leaves. This investigation suggests that the methanolic extracts of *Mollugo nudicaulis* possess potential antioxidant and antibacterial compounds.

50. Sundaraj Rajamanikandan 2012, reported protective effect of *Mollugo nudicaulis* Lam. on acute liver injury induced by perchloroethylene in experimental rats. *M. nudicaulis* possess hepatoprotective and antioxidant activities against perchloroethylene-induced hepatotoxicity in rats.

51. Nagesh and Shanthamma, 2012, studied wound-healing activity of *Mollugo nudicaulis* in Wistar albino rats: Field-grown leaf versus *in vitro*–derived leaf calli extracts. An increase in hydroxyproline content was also observed in the calli extract–treated groups.

52. Naczk, M.; Shahidi, F., 2004. Extraction and analysis of phenolics in food. J. Chromatogr. A. 1054, 95–111.

53. Bajkacz, S., Baranowska, I., Buszewski, B., Kowalski, B., Ligor, M., 2018. etermination of Flavonoids and Phenolic Acids in Plant Materials Using SLE-SPE-UHPLC-MS/MS Method. Food Anal. Methods. 11, 3563–3575

54. Catalán, Ú., Barrubés, L., Valls, R.M., Solà, R., Rubió, L., 2017. In vitro Metabolomic Approaches to Investigating the Potential Biological Effects of Phenolic Compounds: An Update. Genom. Proteom. Bioinform. 15, 236–245.

55. Ronald M.A Knegtel, Irwin D Kuntz, C.M Oshiro, Molecular docking to ensembles of protein structures11Edited by B. Honig, Journal of Molecular Biology, Volume 266, Issue 2, 1997, 424-440

56. Douglas, B. K., Helene, D., John, R. F., Jurgen, B., 2004. Docking and scoring in virtual screening for drug discovery: methods and applications. Nat rev. 3, 935-949.

57. Meng, X., Zhang, h., Mezei, M., Cui, M., 2011. Molecular Docking: A powerful approach for structure-based drug discovery. Journal of Current Computational Aided Drug Research. 7(2), 146-157.

58. Islam, M.N., Ishita, I.J., Jung, H.A., Choi, J.S., 2014. Vicenin-2 isolated from Artemisia capillaris exhibited potent anti-glycation properties. Food Chem Toxicol. 2014; 69:55–62.

59. Singhal, S.S., Jain, D., Singhal, P., Awasthi, S., Singhal, J., Horne D., 2017. Targeting the mercapturic acid pathway and vicenin-2 for prevention of prostate cancer. Biochim Biophys Acta Rev Cancer. 1868 (1), 167-175.

60. Sun, D. W., Zhang, H. D., Mao, L., Mao, C. F., Chen, W., Cui, M., 2015. Luteolin inhibits breast cancer in vitro and in vivo by suppressing notch signaling and regulating MiRNAs. Cell. Physiol. Biochem. 37, 1693–1711.

61. Zhang, B. C., Zhang, C. W., Wang, C., Pan, D. F., Xu, T. D., and Li, D. Y., 2016. Luteolin attenuates foam cell formation and apoptosis in Ox-LDL-stimulated macrophages by enhancing autophagy. Cell. Physiol. Biochem. 39, 2065–2076.

62. Brown, J. E., Rice-Evans, C. A., 1998. Luteolin-rich artichoke extract protects low density lipoprotein from oxidation in vitro. Free Radic. Res. 29, 247-255.

63. Chen, G.; Li, X.; Saleri, F.; Guo, M. Analysis of Flavonoids in *Rhamnus davurica* and Its Antiproliferative Activities. *Molecules* 2016, *21*, 1275.

64. Dharambir, K., Ajay, S., Hardeep, S., Katrin, S., Sandeep, P., Tapan, K., 2017. Kaempferol – A dietary anticancer molecule with multiple mechanisms of action: Recent trends and advancements, Journal of Functional Foods. 30, 203-219.

65. Liu, Z. K., Xiao, H. B., Fang, J., 2014. Anti-inflammatory properties of kaempferol via its inhibition of aldosterone signaling and aldosterone-induced gene expression. Canadian Journal of Physiology and Pharmacology. 92(2), 117–123.

66. Maddox CE, Laur LM, Tain L. Antibacterial activity of phenolic compounds against the phytopathoger Xylella fastidiosa. Curr Miccrobiol. 2010;60:53e58.

67. Jeyadevi R, Sivasudha T, Ilavarasi A, Thajuddin N. Chemical Constituents and Antimicrobial activity of Indian Green Leafy Vegetable Cardiospermum halicacabum. Indian J Microbiol. 2013;53:208e213.

68. Kiyama H, Oono T, Huh WK, et al. Actions of farnesol and xylitol against Staphylococcus aureus. Chemother. 2012;48:122e128.

69. Jabra-Rizk MA, Johnson JK, Forrest G, Mankes K, Meiller TF, Venezia RA. Prevalence of Candida dubliniensis Fungemia at a large teaching hospital. Clin Infect Dis. 2005;41:1064e1067

70. R. Patil, S. Das, A. Stanley, L. Yadav, A. Sudhakar, A. K. Varma, Optimized hydrophobic interactions and hydrogen bonding at the target-ligand interface leads the pathways of drug-designing, *PLoS One*. 5 (8) (2010) e12029.