Final Report (Group)

INNOVATIVE PRODUCTS DESIGN AND DEVELOPMENT TO COMBAT COVID-19 AND RELATED INFECTIOUS DISEASES

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1. Background

COVID-19 is the defining global health crisis of our times and is one of the greatest challenges we have faced since World War II together with pulmonary tuberculosis and AIDS[1]. After its emergence in Asia at the end of 2019[2], it has expanded rapidly throughout the world as a pandemic and has resulted in 446 million infections and loss of more than 6 million individuals[3]. Nepal is highly vulnerable to this unfolding and novel catastrophe with nearly 1 million infections and 12,000 deaths[3]. The Government of Nepal has enforced multiple nationwide lock downs and safety protocols have been established along with the activation of federal, provincial and local level mechanisms to respond to the crisis[4]. It has been evidenced during the times of the lock down that our preparedness for the response through materials, medicines and manufacturing of essentials products has not been prompt and adequate[5]. The administration of vaccines has provided some immunity but covering a significant proportion of the population would be time consuming. As of 7 Mar, 2022 about 64% of the global population are vaccinated with at least a single dose[6]. Meanwhile, the secondary impact (economic, mental, social, health-care, academic etc.) of this pandemic is huge and is resulting in serious consequences effecting every aspect of life[7]. Also, in our country, it is expected that quite a worse scenario might prevail in context to the unavailability of fundamental techniques for rapid and reliable detection and confirmation of the infections in broad socio-demographic population. The halting of this air borne virus with adequate protection and overall compliance of guidelines by the public are deemed the utmost factors to contain the spread of the disease.

It seems that we have to urgently plan and prepare our own preventive system to combat this pandemic and for the uncertain future as well. We have a challenge to tackle, that is to develop the named materials and techniques of high quality in low resource setting that can be commercialized instantly by Nepali entrepreneurs ensuring the efficacy of product and its safety.[8] We propose an interdisciplinary and inter-institutional collaborative research project targeting a much anticipated prompt inhibition of the accelerating viral infections. A major hurdle in Nepal for COVID-19 management is that of the poor availability of personal protective equipment (PPE) and diagnostics tools[9]. Hence by the development of functional, high quality health care and diagnostics accessories at the local level, these shortcomings can be properly addressed. This will help to curtail the infections of the current pandemic and forms the basis for the timely control and adequate response to any future outbreaks.

PPE have become an important and sensitive material during the current pandemic. COVID-19 is predominantly caused by human contact or oral droplet transmission and is attributed to relatively large respiratory particles which are subject to gravitational force and travel only approximately one meter from the patient[10]. Airborne transmission may occur if the patient's respiratory activity produces a respiratory aerosol. These aerosols can travel certain distance and remain airborne for long periods of time, but their infectious potential is uncertain. Therefore, using personal protective materials to prevent infection is a very important and an effective preliminary safety measure. Proper use of such materials would reduce the risk of viral transmission. Example of such material include high-filtration masks and is being currently under scientific investigation[11].

On the background for need on innovative technologies development towards the nation's aspiration on sustainability/prosperity journey and the present context of COVID-19, we propose the interdisciplinary research and development (R&D) activities. The initiation will be useful not only for developing innovative technologies and products to address the current needs of the country but also in strengthening research and innovations in materials science and nanotechnology. The research in biomedical fields offering internationally competent research, multidisciplinary approaches and emphasis on new innovations with inter-institutional collaboration is deemed to be appropriate. The scientific research with local resources (plants) that can provide immediate results and that is sustainable on the long run seems worthy and justifiable.

Natural fibers like allo, argali, bamboo, hemp, banana, jute, Spanish dagger, and orka are low-cost sustainable materials readily available in Nepal. They are sturdy, light-weight, biodegradable, renewable and possess desired and tunable properties. These fibers are made up of different components like cellulose, hemicellulose, lignin, waxes, pectin etc and are present in varying amounts. These components along with other materials like alginate, polyvinyl alcohol, carboxy-methyl cellulose, methyl cellulose etc can be utilized individually or as composites owing to their high surface exposure and mechanical strength for various purposes. Particularly, the materials forming layered morphology are interesting in terms of diverse properties that can be easily tuned and controlled according to the requirement by soft laboratory procedures. One of the reasons of considering the natural fibers is in their role as a substrate with air permeability for holding the selected phytochemical (LIB_RECAST)[12] in the development of product for combating viral or other related diseases.

A ligand with proper composition, size, geometry or orientation and the functional groups can possess good binding affinity at the active or allosteric site required for halting or decelerating the regular functioning of protease. The involvement of the amino acid residues in the interaction with the ligand in the docked pose along with hydrogen and polar/electrovalent bonding that disrupts the regular function of the protease has to be found for systematic understanding of the whole therapeutic process.

In this work, eucalyptol (ECL, $C_{10}H_{18}O$), piperitone (PPT, $C_{10}H_{16}O$) and methyl-(e)-cinnamate (MEC, $C_{10}H_{10}O_2$) has been studied as antiviral compounds (more in phytochemical section) with druglikeness (Lipinski, Veber and Egan)[13] that can inhibit SARS-CoV-2[14]. The relevance and effectiveness of selected phytochemicals in combating the virus requires molecular docking and dynamics simulation studies to reveal the atomic level interactions. This can provide insights into the role of small molecules in binding with the active or allosteric sites of the selected proteins chosen as therapeutic targets and in evaluating the preventive nature of the compound..

Among 29 associated proteins[15] of SARS-CoV-2, five from different domains each with its distinctive function have been considered as suitable targets for the proposed drugs. The structures[16] of these receptors have been published and corresponding PDBIDs (RCSB database) for molecular docking and dynamics studies are shown in Table 1.[17]

Table 1: Different receptors/domains of SARS-CoV-2 and the proteins

Receptors/Domain of the Virus	PDBID
Spike protein S1 (S)	7A92 (Chain A)
Main protease (M^{pro})	7CB7 (Chain B)
Papain-like protease (PL^{pro})	7CJM (Chain A), 7SQE[18]
Envelope protein (E)	7M4R (Chain A)
Angiotensin-converting enzyme 2 (ACE2)	7RPV (Chain A)

The Spike protein S (S1) is involved in virus attachment, fusion and entry into host cell. Angiotensin-converting enzyme 2 (ACE2) in the host is one of the receptors for the entry. Main protease (M^{pro}) helps in transcription and replication in host cell by polyprotein processing. PL^{pro} is an essential coronavirus enzyme (Nsp3, nonstructural protein) that not only aids in processing viral polyproteins to create a functional replicase complex which eventually helps in spreading but also in disrupting the

antiviral immune responses.[19] Here two Nsp3 proteins with PDBIDs of 7CJM and 7SQE have been chosen due to difference in their cavity sizes at the active site. Envelope protein (E) interacts with membrane protein to form viral membrane before exocytosis. All these distinct functions are depicted diagrammatically in Figure 1 in viral life cycle.

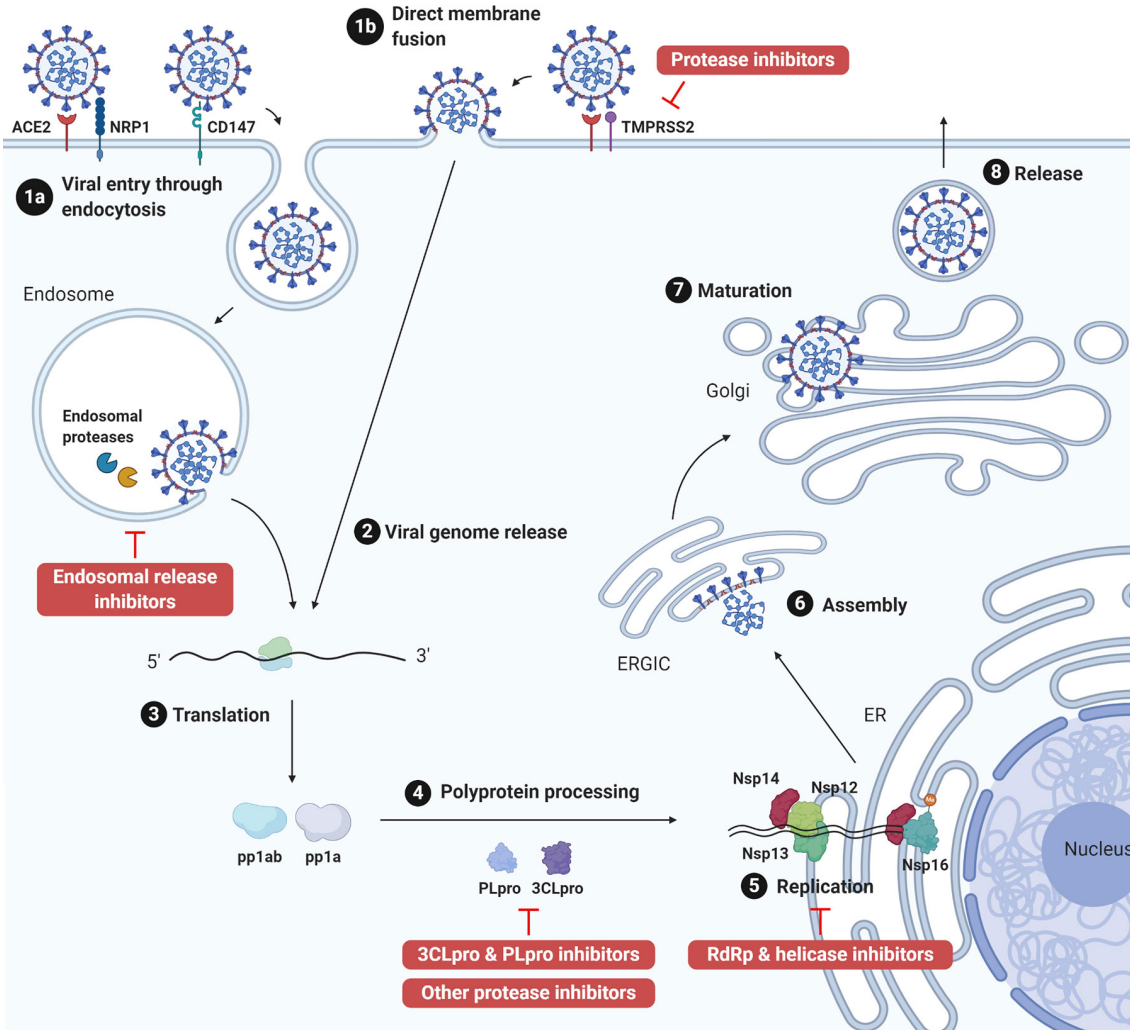


Figure 1: Life cycle of SARS-CoV-2 and selected inhibitory targets[20]

Since each chosen target has a distinct function, if any one or more of these receptors are strongly docked with a suitable phytochemical, then the inhibition of the virus is likely. A small disruption in the viral life cycle phases would lead to the deceleration of its spread. The *in silico* approach aims to study the possibilities of binding of locally available phytochemicals to active sites of various proteins with favorable orientation and stability that would eventually helps to fight with the virus. The target receptors are shown as ribbon models in Figure 2.

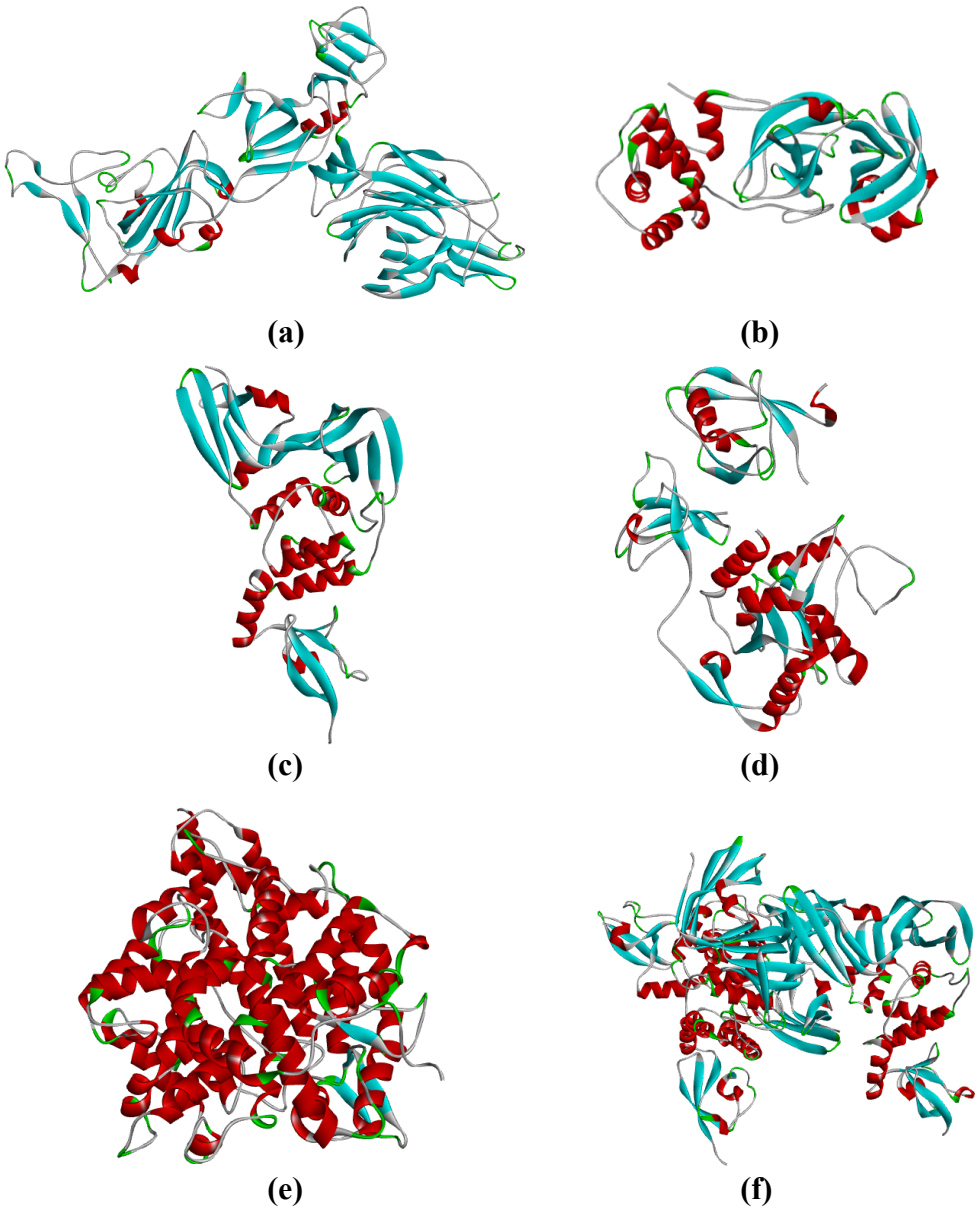


Figure 2: Six target proteins as receptors of ligands with PDBID (a) 7A92 (b) 7CB7 (c) 7CJM (d) 7M4R (e) 7RPV and (f) 7SQE; alpha helix in red, beta sheets in cyan and loops/turns in grey

1.1 Rationalization of the Selection of Suitable Natural Fiber Substrates

Material selection for designing and manufacturing a product plays a vital role in the field of engineering design. Thus, cellulose-based natural fibers were selected as the starting substance to achieve superior performance. Nowadays, natural fibers have received revived attention as wide acceptance materials for their biodegradability and lower density compared to artificial fibers[21,22]. Also, these fibers have inherent mechanical and thermal properties suitable for our work[23]. Therefore, pulp obtained from fibers was used to fabricate suitable substrates. Surface functionalization of fibers was of good importance as it widens the application perspective. This chemical modification augments the absorption capacity by increasing the number of active sites in comparison to that of the bare wood pulp. In addition, the fiber pulp was found to possess greater tear resistance and tensile strength that assured structural integrity and thermal stability of the product[24].

1.2 Phytochemicals and Significance

Phytochemicals have been also a source of medicine for providing resistance against and curative properties since time immemorial. There are several components of bioactive compounds in medicinal plants used in Ayurvedic preparation which play important role in remedial effect combating epidemic diseases. The herbal exports from Nepal are in the form of raw materials collected from the wild and some are cultivated. More recently, the Department of Plant Resource (DPR) has developed a Material Safety Data Sheet (MSDS) for 20 essential oils produced and exported from Nepal. This type analysis not only provide initial steps for safeguarding a sustainable market for farmers, collectors and processors but also provide opportunity for product development which can make contribute in management of respiratory diseases. The recommendation by the State Administration of Traditional Chinese Medicine of China and extensive use of traditional Chinese medicine in integrated way contributed in handling of COVID-19 situation in very effective way in China.[25] Some leading centers in Nepal working in the field of medicinal and aromatic plants are Jadibuti Association of Nepal (JABAN), the Ayurvedic Medicine Producers Association of Nepal (AMPAN), the Herbal Entrepreneur Association Nepal (HEAN), the Nepal Herbs and Herbal Products Association (NEHHPA) and the Federation of Community Forestry User's Nepal (FECOFUN). However, there is limited use of essential oil produced within Nepal and even lesser for medicinal purpose.

Exports of essential oils from Nepal are relatively small, but are on the rise. Between 2010 and 2015, exports of essential oils rose by a compound annual growth rate of 11% from US$ 974 to 1,626 thousand. By volume, exports rose from 21 to 37 tons. The Nepalese herbal material supplied to India and China includes 300 species[26] and the annual export has been estimated to be between 7,000 and 27,000 tones at a value of USD 11 to 48 million (in 2020 value, inflation-adjusted figures)[27,28]. The best opportunities for MAPs and oils from Nepal are in three key segments: premium flavour (natural health food and organic food), premium beauty and personal care products and Pharmaceutical (herbal traditional medicines).[29] Some potential Nepalese products such as Black cardamom (*Amomum subulatum*), Soti grass (*Cymbopogon jwarancusa*) and Sugandha kokila (*Cinnamomum glaucescens*) have immense possibility of development of essential oil based products for combating respiratory diseases.

There had been some effort in herb cultivation and processing in Nepal but significant herbal material collected from Nepal are either sent to India and China or other countries and whenever those countries stop importing herbal material gets dumped in Nepal. There is a dire need and responsibility of identification, prioritization, conservation, propagation, cultivation, processing and marketing of Nepalese products.[30] There is a need of attaining international accreditation and international recognition of test results that require sustained commitment and investment over many years (World Bank, 2018) for utilization of Nepal's own products.

1.3 Plant materials

1.3.1 Black cardamom (*Amomum subulatum*)

Amomum subulatum belongs to the family Zingiberaceae. It is commonly known as black cardamom and as 'Alainchi' in Nepali. It is a perennial crop native to Nepal including some regions of India and Bhutan. Nepal is the largest producer of the plant, around 52% of the total world production is produced in Nepal. The black cardamom is a high value cash crop grown predominantly in Eastern Nepal mainly in Taplejung, Panchthar, Ilam and Sankhuwasabha. The annual production of Nepal exceeds 6,600 Metric Ton and more farmers are getting involved in expansion of cultivation.[31]

The fruit of the plant is used as a spice and as a flavoring agent in confectionary, and beverages due to its pleasant aroma. Traditionally, it has been used as a medicinal plant in the treatment of various ailments like digestive disorder, cough, throat infection, wounds, inflammation of eyelids, diarrhea,

skin diseases, congestion of lungs and more[32] The composition of essential oil of the plant varies depending upon variety, region, and on the maturity of the fruit. The black cardamom seed from Nepal is known to contain 1,8-cineole 60.8%, α-terpineol 9.8%, β-pinene 8.3% and α-pinene 6.4%.[33] The ethanolic and aqueous extract of fruit is known to exhibit anti-inflammatory activity.[34] The antimicrobial activities are known against *Lactobacillus acidophilus, Streptococcus mutans, Staphylococcus aureus, Saccharomyces cerevisiae*, *Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans.*[35] The increase in production and development of technology in order to utilize the product can provide much needed benefit from black cardamom produced in Nepal.

1.3.2 Soti grass (*Cymbopogon jwarancusa*)

Cymbopogon jwarancusa belongs to family Poaceae. The plant is known as 'Sotigrass' in Nepal and known as 'Jwarankush' referring it as fever breaker in Sanskrit. Traditionally, the plant is used as a medicine for different diseases like fever, vomiting, blood impurities, skin problems, unconsciousness, and abdominal tumors.[36] Various researches have shown presence of antibacterial, antifungal, insecticidal properties of *C. jwarancusa*.

A major compound present in *C. jwarancusa* oil is piperitone. The variation of piperitone among genotypes as 44.9-66.8 % and concentration reaching 68.0% at the time of seed formation have been reported.[37] The presence of bioactive compounds is responsible for different biological activities. The *C. jwarancusa* essential oil had been reported for marked inhibition of the growth *Streptococcus pneumonia, Aspergillus fumigatus* and *Candida albicans.*[38]

1.3.3 Sugandhakokila (*Cinnamomum glaucescens*)

Cinnamomum glaucescens belongs to family *Lauraceae*. It is known as 'Sugandhakokila' in Nepal. The plant is also reported from some hilly region of India and Bhutan. It is one of the essential oils producing plants of Nepal with commercial value. The plant is famous as a spice and medicine among the local people (Satyal et al., 2013). Traditionally, the seed of the plant is used as medicine for the treatment of common cold, cough, toothache, joint pain, and body aches.[39]

The major bioactive compound present in the seed oil of *C. glaucescens* were 1,8-cineole and methyl cinnamate while sabinene, terpinen-4-ol, and linalool were also present in smaller amount.[40] There are reports of nematicidal, termiticidal, mosquito larvicidal, insecticidal, antifungal, antiaflatoxin, antioxidant and antibacterial activities of essential oils of *C. glaucescens.* The essential oil of *C.*

glauscens can inhibit of the growth and aflatoxin production by toxigenic strain of *Aspergillus flavus* and its non-mammalian toxicity is LD_{50} (3971.34 µl/kg) during oral toxicity.[41]

2. Objectives/ Goals

General Objective- Considering the current epidemic situation and the uncertainty it has created in all the possible sectors, there is an urgency in its quick control. Targeting this point, the broad objective of the work would be to design and develop innovative, instantly wearable and readily available antiviral medicated health care products to combat COVID-19 and related infectious diseases.

Specific Objectives- In order to meet the above mentioned general objective, following are the multiple specific objectives of this project

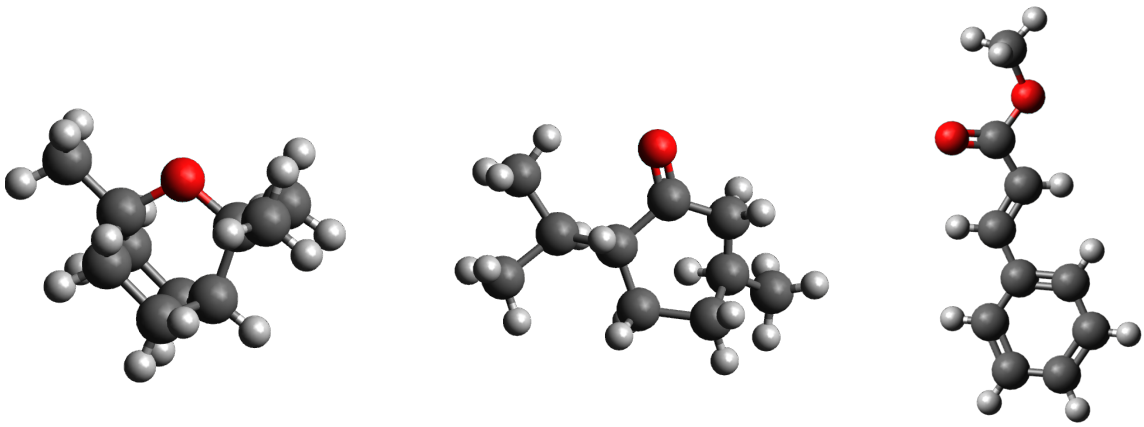
a. *In silico* atomic level assessment of the phytochemical as potential therapeutics against target receptors of different domains of virus (molecular docking and molecular dynamics)
b. Development of natural fiber based substrate with better adsorption and mechanical capabilities from more than eight plants
c. Preparation of essential oil containing phytochemicals from readily available plants (three chosen for this study)
d. Development of prototype of protective equipment (medicated face masks)

3. Results and discussion

The phytochemicals used in this research being low molecular weight compounds, possess good volatility capable of being used in face masks and also of being adsorbed in the fabric used. To add another value to this system, its probabilistic inhibitory role in stopping the virus has to be studied first to come any conclusion. In this course, the details of the output from computational studies are presented next.

3.1 Computational outcomes

The energy minimized and force relaxed molecular structures of the ligands were obtained from semi-empirical calculations. The docked complexes with the receptor proteins at orthosteric or allosteric sites were determined after blind docking. Here two compounds, hydroxychloroquin (HCQ, $C_{18}H_{26}ClN_3O$) and ribavirin (RBV, $C_8H_{12}N_4O_5$) are taken as standard FDA drugs for comparative analysis. The optimized molecular structure of the phytochemicals are shown in Figure 3.



(a) **(b)** **(c)**

Figure 3: Optimized molecular structure of phytochemicals
(a) Eucalyptol (b) Piperitone and (c) Methyl-(E)-cinnamate;
carbon in dark, oxygen in red and hydrogen in light grey

The scoring of the best pose of the ligand expressed in terms of binding affinity (kcal/mol) was sorted out and the lowest one denoting the most favorable binding is shown in Table 2 for each receptor.

It was found from blind docking that in all the three cases the same active site of the protease was involved even with different input parameters proving that no other allosteric sites were effective in binding the phytochemicals efficiently. The docking poses were further finely searched in small volume around the active site after blind docking for obtaining better resolution and orientation. The binding affinities of the three ligands and the interacting amino acid residues that hold the ligand in the cavity is displayed in Table 2. The absence of hydrogen bonding or other electrostatic interactions in these complexes yield higher values and is attributed to the lack of minimal hydrogen bond acceptors or donors in these molecules (Table 3). This points towards the selection of compounds with larger number of hetero-atoms or chain branching with diverse functional groups that can take part in hydrogen bonding.

Table 2: Binding affinities (kCal/mol) of ligands and interacting residues with PL^{pro}(PDBID:7SQE)

Ligands	Binding Affinity	Interacting amino acid residues (within 5 Å)
ECL	-6.4	D164, D166, M208, A246, P247, P248, Y264, N267, Y268, Y273, T301
PPT	-6.3	D161, D164, Q195, Q196, M208, P247, P248, Y264, G266, N267, Y268, Y273, T301
MEC	-6.4	G163, D164, V165, Q195, T197, P247, P248, Y264, N267, Y268, Y273, T301

The binding affinities for the reference compounds was calculated to be -7.1 kcal/mole or lower, implying that these drugs have better binding capacities than the selected phytochemicals for the chosen receptor protease. It is a result of higher molecular weights and presence of many electronegative elements in these drugs that strengthen the interactions with the amino acid residues. Moreover, the root mean square deviation (molecular dynamics simulation) of ligand inside the receptor suggests that there exists considerable motion. It hints at the unstable nature of the complex and reduced inhibitory effect. The case might not be the same with other receptors and the findings will be dealt in next.

Table 3: Hydrogen bond and polar neighbor distances (Å) in different ligand-7SQE complexes

	PPT	ECL	MEC
Hydrogen bond	none	none	none
Polar neighbors	2.3, 2.4 (2), 2.6 (2), 2.8, 2.91.9, 2.2, 2.4, 2.5 (2), 3.2 (2), 3.3 (2), 3.4 (3), 3.5	2.9, 3.2, 3.3, 3.4, 3.5 (2), 3.0 (2), 3.2 (2), 3.3 (2),2.6, 2.9, 3.0, 3.1, 3.2 (2), 3.3 (2), 3.4 (9)	

The preliminary results with 7SQE receptor (with high RMSD) pointed to change of ligands or receptors to ensure stability of the docked complex. As the ligands possess better properties and serves some of the purposes, the alternative of selecting new targets were considered appropriate taking into account the limitations of time and resources. The therapeutic value and efficacy of a ligand can be studied using any target protein as any preference or effectiveness to only one kind has not been fully investigated or established. Hence, results with five different domains of virus that were chosen for *in silico* work for broader assessment and for understanding the role of multiple proteins are presented.

Table 4: Binding affinites (kcal/mol) of different ligands with different proteins

	7A92	7CB7	7CJM	7M4R	7RPV
ECL	-5.6	-6.3	-5.2	-4.8	-5.6
MEC	-5.7	-6.6	-5.7	-5.5	-6.6
PPT	-5.6	-6.3	-5.2	-5.1	-5.5
RBV	-6.3	-6.3	-6.4	-5.9	-6.7
HCQ	-5.9	-6.7	-7.0	-5.7	-6.5

The values for MEC are better (Table 4) than that for the other two phytochemicals in all the five receptors. Hence, future trials with this compound as an effective therapeutic candidate for fighting COVID-19 is recommended. However, it has distributed values in comparison to that of the reference compounds suggesting similar preventive efficacy with the benefit of it being suitable for use in face masks.

The protein structures are not rigid frameworks as they change or adopt to additional interactions that are present in the host due to ligands, solvent, ions, changes in temperature, physiological conditions etc. To account for this intrinsic flexibility, different models of the protein structures were generated using CABS-flex online server.[42] Here the spatial resolution of the generated models (trajectory clustering by k-medoids method) enables the reconstruction of all-atom representation of real structures. For each protein structure, ten different models were created and subjected to docking with MEC ligand. The pose with the best binding affinity in each of the ten cases are shown in Table 5.

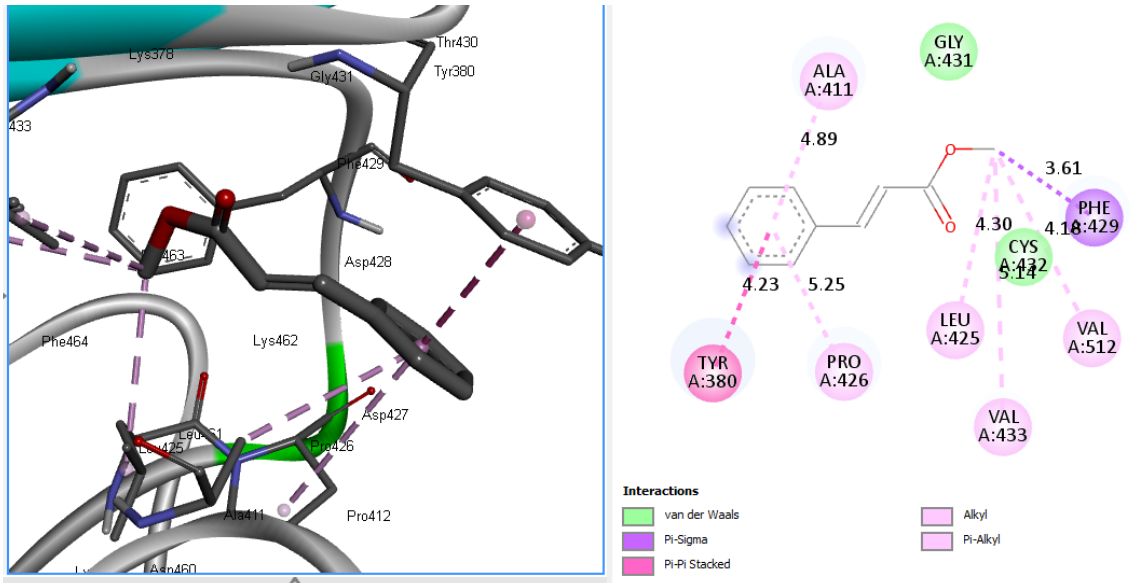
Different active site was selected in case of receptor with PDBID of 7CB7 due to the possibility of better interactions at alternate site in the protein and that the increase of search space would provide reliable docking calculations.

Table 5: Best binding affinity (kcal/mol) of MEC ligand with different flexible models of different proteins (only the top case)

	7A92	7CB7	7CJM	7M4R	7RPV
MEC	-6.0	-6.8	-6.9	-5.6	-6.8

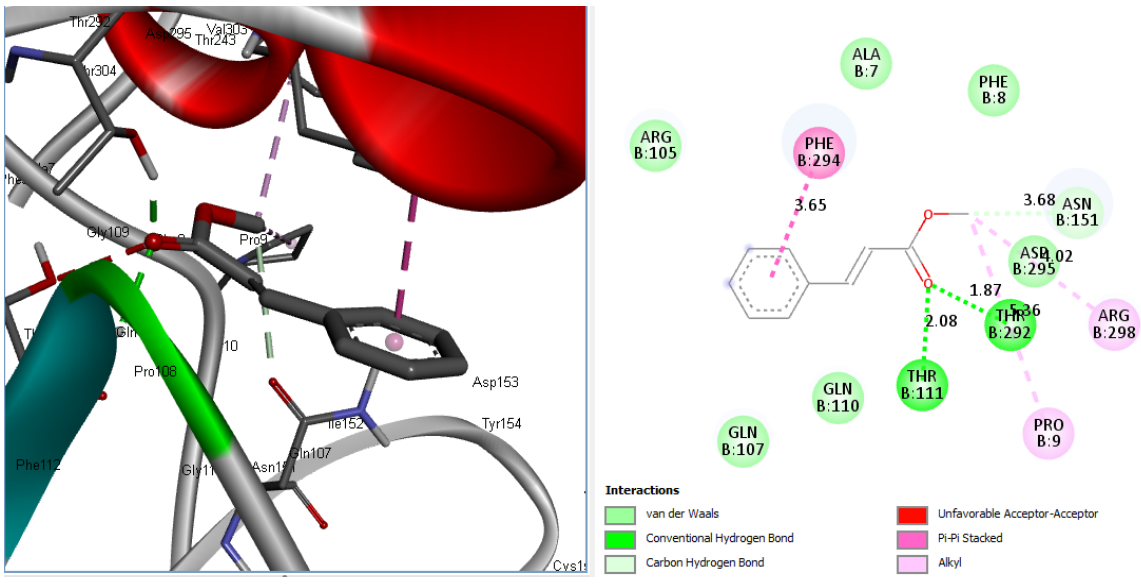
In all five cases, the values become lower (better binding interactions) signifying the importance of adding more degrees of freedom to the search space. Thus the inclusion of protein flexibility is justified as it is one non-trivial biochemical phenomenon that has to be considered during docking studies. Also, these redocked affinities are lower (better) in three of the cases and higher (worse) by 0.1 kcal/mole in two cases relative to that of HCQ. Here the binding affinities of MEC with ACE2, M^{pro} and PL^{pro} is distinctly the top cases and suggests the inhibition of entry point in the virus (ACE2) and disruption of its functioning especially the transcription, replication and maturation (M^{pro} and PL^{pro}).[43] he different types of inhibitors to M^{pro} have been proposed by recent publication using *in silico* methods.[44] Also a review article focusing on PL^{pro} inhibition using different PDBIDs by various molecules have been published.[45] The envelope and the spike proteins do not show promising results and may be discarded since the non-ceasing mutations especially at the spike anyway render the drug ineffective with the passage of time and viral generations. However, using the structure of envelope protein derived from homology modeling, more than 4000 phytochemicals have been reported for evaluating their inhibitory roles.[46] Also, the computational investigation of phytochemicals of different chemical classes from different Indian and Chinese medicinal plants have been published that have been docked with human ACE2 and the spike proteins.[47]In case of comparison with that of RBV, a distributed pattern is observed and thus it can be inferred that MEC stands as a better candidate that can be taken as a hit candidate in the development of this phytochemical as a therapeutic agent. Here, the role of flexible proteins in docking the reference compounds and other two phytochemicals were not accessed and requires full phase *in silico* studies as extended research work. However, this aspect might be included in the final article publication.

The best docked poses and relevant interactions of different cases with flexible protein models are shown in Figure 4-8. The distances are in Å and different color codes are used for distinguishing the interactions between the ligands and amino acid residues.



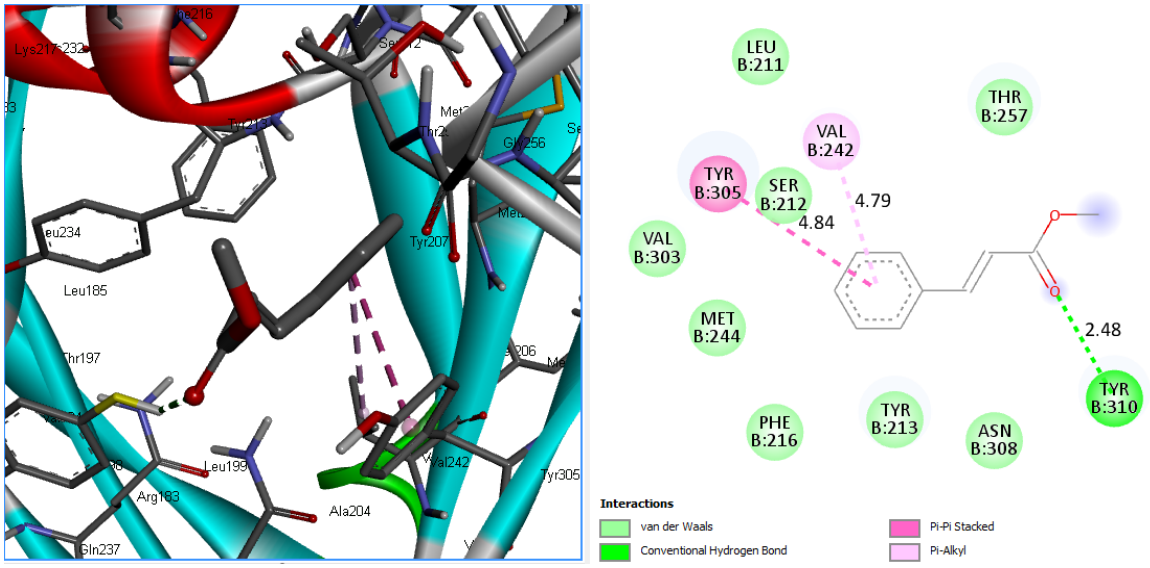
(a) (b)

Figure 4: (a) Interaction of MEC with the amino acid residues in the pocket of receptor 7A92 (b) Different types of ligand-amino acid residue interactions



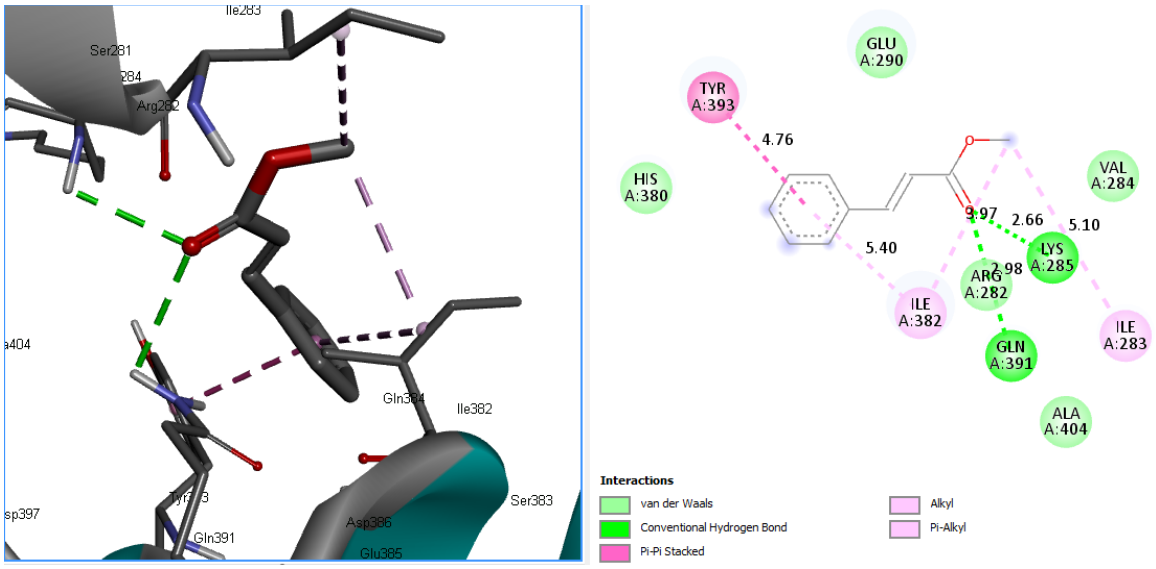
(a) (b)

Figure 5: (a) Interaction of MEC with the amino acid residues in the pocket of receptor 7CB7 (b) Different types of ligand-amino acid residue interactions



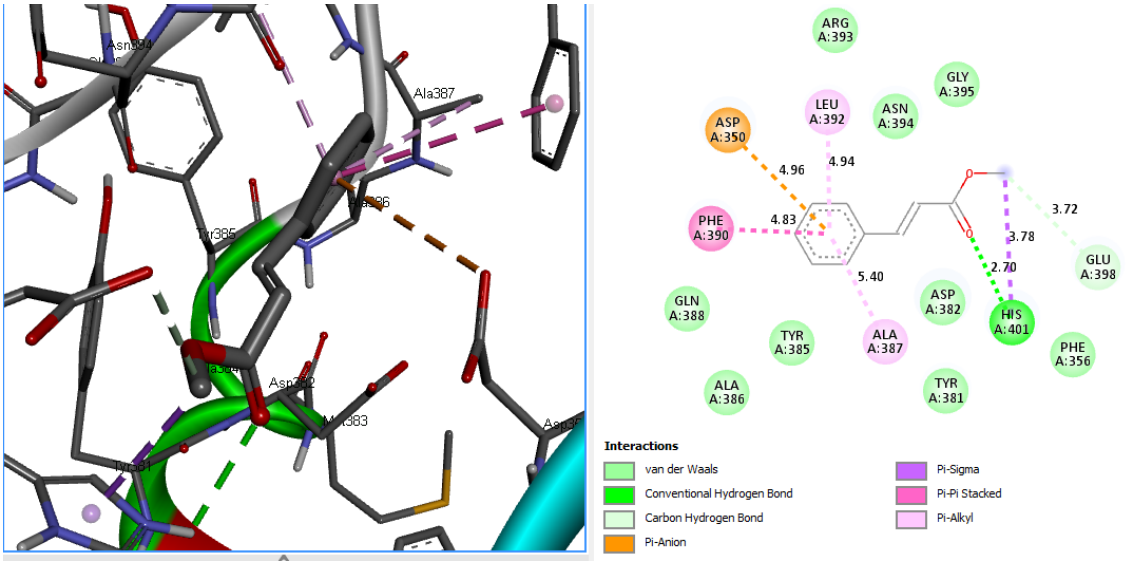
(a) **(b)**

Figure 6: (a) Interaction of MEC with the amino acid residues in the pocket of receptor 7CJM (b) Different types of ligand-amino acid residue interactions



(a) **(b)**

Figure 7: (a) Interaction of MEC with the amino acid residues in the pocket of receptor 7M4R (b) Different types of ligand-amino acid residue interactions



(a) **(b)**

Figure 8: (a) Interaction of MEC with the amino acid residues in the pocket of receptor 7RPV (b) Different types of ligand-amino acid residue interactions

Due to the presence of hydrogen bonding, pi-pi stacked and alkyl related interactions in nearly all the cases, the ligand is properly bonded at the active site of the protein. The spike protein lacks the hydrogen bonding and binding affinity shows relatively weaker interactions. There are no significant unfavorable interactions present except in the protease 7CB7 (Figure 5) where acceptor-acceptor distance of 2.801 Å tends to destabilize the complex. Maximization of ionic bonding and electrostatic interactions seems to favor the binding affinity. The number of hydrogen bond donors and acceptors are low in this set of phytochemicals and considering this feature as primary selection criteria in choosing ligands might improve the scoring and docking in future work. The molecular dynamics simulations showed that the docked poses are not completely stable and requires elongated time spans in the range of hundreds of nanoseconds to come to a definite conclusion. It will be pursued as future work along with free energy calculations. The interacting residues with the types of interactions with MEC are summarized in Table 6.

Table 6: Different types of interactions of MEC ligand with the residues in the receptors

	7A92	7CB7	7CJM	7M4R	7RPV
Hydrogen bond	-	Thr111, Thr292	Tyr310	Lys285, Gln391	His401
Pi-pi stacked	Tyr380	Phe294	Tyr305	Tyr393	Phe390
Pi-sigma	Phe429	-	-	-	His401
Alkyl/Pi-alkyl	Ala411, Leu425, Val433	Pro426,Pro9, Arg298 Val512,	Val242	Ile382, Ile283	Leu392, Ala387

3.2 Phytochemical Results and Analysis

The samples of Black cardamom (*Amomum subulatum*) of Nuwakot and Panchthar were processed using Soxhlet extractor gave 5.02 % and 4.01% oil yield in case of *n*-hexane extract and use of Clevenger apparatus for hydrodistillation gave 2.79 % and 3.20 % essential oil (Table 1). Thus obtained essential oils were placed in -4 ^{o}C until use in experiential purpose. The hydrodistillation of sample of Soti grass (*Cymbopogon jwarancusa*) from Karnali, Western Nepal gave 1.2% yield and Sugandhakokila (*Cinnamomum glaucescens*) from Dang gave 2.5% yield. (Table 1)

Table 1. Yield of essential oils from different species and parts

S.N.	Species	Part	Extract % w/w
1.	Amomum subulatum Black cardamom, Alainchi	Seed	Hydrodistillation extract Nuwakot sample 2.79 % Hydrodistillation extract Panchthar sample 3.20 % *n*-hexane extract Nuwakot sample 4.01 % *n*-hexane extract Panchthar sample 5.02 %
2.	Cymbopogon jwarancusa Soti grass	Leaf	Hydrodistillation extract Karnali sample 1.2 %
3.	*Cinnamomum glaucescens* Sugandhakokila	Fruit	Hydrodistillation extract Dang sample 2.5%

The black cardamom (*Amomum subulatum*) seed and rind are known to contain with 1,8-cineole (60.8% and 39.0%), *α*-pinene (6.4% and 4.8%), *β*-pinene (8.3% and 17.7%), and *α*-terpineol (9.8% and 12.3%) [9]. *Cymbopogon jwarancusa* essential oil is known to contain piperitone, (44.9–66.8%), elemol (7.0–29.2%) and δ-2-carene (8.3-23–5%) in different genotypes.[48] *Cinnamomum glaucescens* essential oil is known to contain methyl-(*E*)-cinnamate (40.5%) and 1,8-cineole (24.8%), α-terpineol (7.4%), sabinene (5.7%), terpinen-4-ol (4.8%), and linalool (3.7%).[16] The essential oils obtained were used for embedding in matrix for preparation of path for application in face mask.

In Nepalese context bamboo represent one of the readily available renewable resources. Bamboo charcoal was prepared by pyrolysis or carbonization possess extraordinary characteristics such as high conductivity, large surface area and efficient adsorption of wide range of materials.[49] The pore characteristics of this material include macropore (>50 nm), mesopore (2-50 nm) or micropore (< 2nm). It can be used for adsorption of essential oil for use in fiber matrix as well as vaporizer. Bamboo split cut into appropriated size were carbonized at 400 ^{o}C an electric muffle furnace for 45 minutes which lead to weight loss of 34 % (Figure 3).

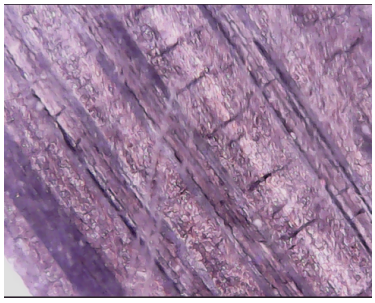
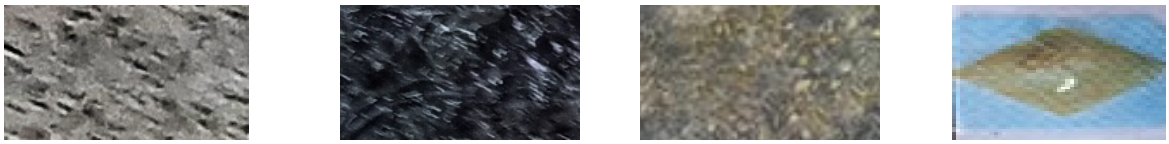


Figure 3. Bamboo charcoal carbonized structure

Plant fibers with distinct diameters and morphologies have excellent wettability and gradual release of absorbed essential oil can be effective antimicrobial capacity.[50] The gelatine based emulsion hydrogels with different proportions of oil can be used for controlled release.[51] Several combinations were tried and among them the combination of twenty grams of gelatin dissolved in 100 ml of water with maintenance of temperature at 50 ºC and with constant stirring at 400 rpm, so as to obtain a homogeneous mixture and addition of 20 gram of polyethylene glycol (PEG) and 20 g of glycerine monosterate together with addition of hemp fiber (20 g) or charcoal 5 g separately as well as in combination (Figure 4). The mixture thus prepared was poured into petri-dishes and were allowed to form gels and used for embedding with essential oils and evaluation of retention capacity.



(a) **(b)** **(c)** **(d)**

Figure 4. Different matrixes prepared for adhesive patch (a) Gelatin, activated charcoal matrix (b) Gelatin, activated charcoal and hemp fiber matrix (c) Gelatin, Polyethylene-glycol and hemp fiber matrix and (d) Essential oil embedded fiber matrix used in adhesive patch

3.3 Limitations

The collection and analysis of samples from different regions and their seasonal variation can provide insight into concerned phytochemical amount in the sample. The availability of laboratory facility for processing of sample in larger quantity can provide opportunity of isolation of targeted compounds which can be further used for bioassay based experiments.

4. Procedure/methodology

4.1 Computational procedure

4.1.1 Preparation of ligands

The 3D structure of ligand molecules were downloaded from PubChem database[52] in sdf format and converted to pdb or xyz formats. After geometry optimization, it was then processed in AutoDock Tools (ADT)[53] by removing non-polar hydrogen atoms, adding gasteiger charges, setting torsional degrees of freedom for rotational bonds and saving as pdbqt files.

4.1.2 Cleaning of receptors

The tertiary/quaternary structure of the target proteins were obtained from RCSB database[54] as pdb format. The solvent/water molecules, ions and ligands were removed using ChimeraX[55] or Pymol software.[56] Polar hydrogens and Kollman charges were added using AutoDock Tools. The absence of non-bonded atoms and broken protein backbone or chain were ascertained. It was saved as pdbqt file.

4.1.3 Molecular docking

The volume of 30 x 30 x 30 $Å^3$ around the active site of receptor, an exhaustiveness of 40 and energy range of 4 were chosen for docking the ligand. The spacing of 0.375 Å was considered except in case of blind docking where larger value was chosen. The center of grid box and its adequate size were chosen for each receptor upon visual inspection. The config file was constructed accordingly and the output was obtained as pdbqt file. AutoDock vina program[57] was used for obtaining best 10 poses of ligand with the receptor.

4.1.4 Generation of different models of proteins

CABS-flex server[58] was used to generate different protein models in the default mode. The choice of the chain was explicitly specified.

4.1.5 Molecular dynamics simulation

The GROMOS force field[59] was used for topology generation for the ligand and the receptor. For this either ATB[60] or PRODRG2[61] servers were used. After equilibrating the system for 300 ps each at NVT and NPT conditions, the production run was carried out for more than 40 ns using GROMACS code.[62] The build-in processing tools were used for data analysis and Discovery Studio Visualizer[63]/VMD[64] for visualization of the complexes and monitoring the trajectory.

4.2 Fiber extraction and processing

4.2.1 Extraction of Natural Fiber and results

Fiber extraction procedures depend on the type, kind of plant, and portion from which the fibers were derived such as bast, leaves, stem, fruit, wood, etc. But the separation of the fibers from the original plant source is always a key step to obtain the high quality of fibers. Natural plant fibers are extracted and processed by various means that include methods like retting, breaking, scutching, hackling, and combing[65,66]. Cellulosic fibers were extracted naturally using bacteria and fungi. Physical, chemical, and mechanical methods were also followed to extract the fiber. All these methods were performed in such a manner that the morphological features were improved, and processing was done in better ways without alteration of the chemical composition[67]. Natural methods involve the process to extract fibers using bacteria and fungi in the environment to remove lignin, pectin, and other substances.[68,69] Following are the steps involved in the extraction of natural fibers.[70]

4.2.1.1 Selection of Raw Fiber Plants

The matured plants were selected as sources of raw fibers and harvesting was done. The selection of plants was made according to the nature of fibers and their yield. The details of the plant sources is presented in Table 7.

Table 7: List of used Fibrous Plants and their Characteristics

S.N.	Common Name	Scientific Name	Fibrous part	References
1.	Allo	Girardinia diversifolia	Stem	[71]
2.	Argali	Edgeworthia gardneri	Stem Bark	[72]
3.	Bamboo	Bambosoideae	Stem	[73]
4.	Hemp	Cannabis sativa	Stem	[74]
5.	Banana	Musa acuminate	Trunk	[75]
6.	Jute	Corchorus olitorius	Stem	[76]
7.	Spanish Dagger	Yucca gloriosa	leaves	[77]
8.	Okra	Abelmoschus esculentus	Stem	[78]

4.2.1.2 Retting

Retting is the most common technique for the extraction of fibers from different plants by natural methods. Bacteria and fungi play a significant role in retting that breaks the chemical bonds of pectin that hold the stem together and allows the separation of the fibers from the remaining parts. Water retting involves immersing fiber bundles in clear slow-flowing water in canals, rivulets, or ponds[79,80]. Bacteria retting are based on the natural action of anaerobic bacteria, aerobic fungi. Bacillus and Clostridium bacteria are used in water retting while *Rhizomucor pusillus* and *Fusarium lateritium* fungi

are used in dew retting.[81,82] In some cases, alkalies, mild acids, and enzymes are used for fiber extraction. Sodium hydroxide is the most used chemical for fiber extraction.

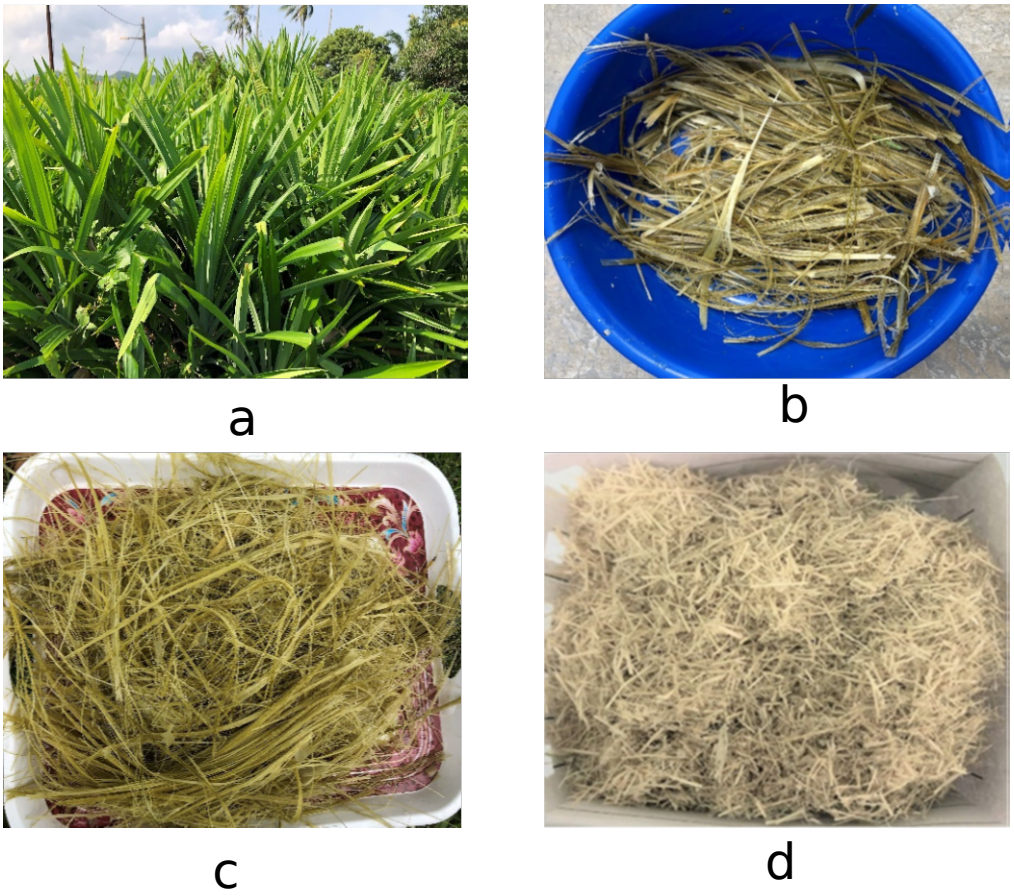


Figure 9: Pictorial view of fiber extraction (a) Yucca gloriosa plant (b) leaves cleaned and immersed in the water for 3 weeks; skin degraded and removal of fibers (c) extracted fibers collected and dried under sunlight (d) ground fibers (short fibers afters being cut by a mechanical grinder)

Initially, bundles of source materials were cleaned and chopped into 2 cm - 3 cm pieces. Then the materials were then placed in stagnant water to undergo water retting for 4 weeks. Throughout the process, it was observed that the freshly collected raw materials were green in color at the beginning and changed to yellow-brownish color after 4 weeks of water retting. The retted leaves were washed in running water and the fibers were removed by manual peeling. The extracted fibers were then cleaned and allowed to dry under direct sunlight for 1 day. Finally, the fibers were ground using a mechanical grinder to obtain short sized fibers form. The fibers were kept in the zip-locked bag until further use.

The images of different steps during the retting process of a representative plant (*Yucca gloriosa*) are shown in Figure 9.

4.2.1.3 Extraction

The fibers were placed in a Soxhlet extractor (Figure 10) and solvent extraction was done using toluene: methanol: acetone in the ratio of 4:1:1 (v/v) within 3 hours.[83] The samples were then dried in a hot air oven for 24 h at 110 °C.

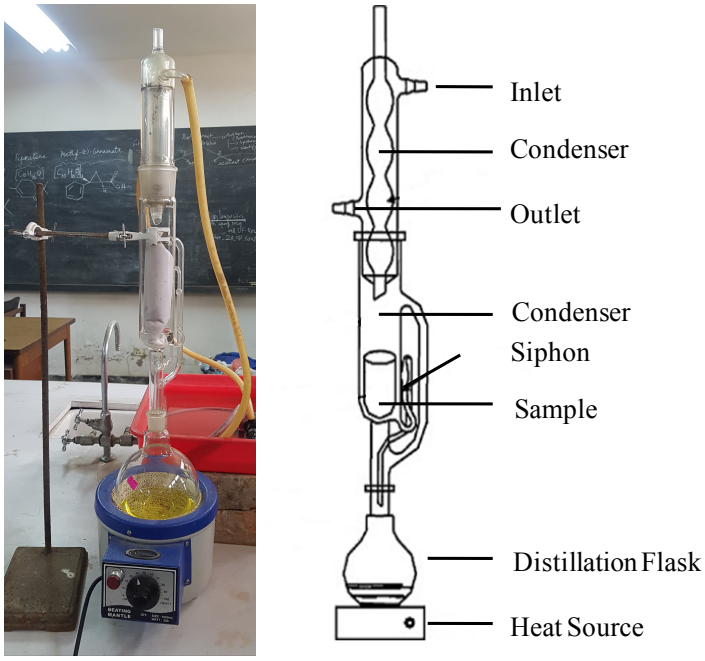


Figure 10. Pictorial representation and Schematics of Soxhlet extraction

4.2.1.4 Bleaching

Different raw fibers were bleached by sodium hypochlorite solution (1.7 wt% $NaClO_2$ in water) at 80 °C for 6 h as shown in Figure 11. After bleaching a desired level of whiteness was achieved. Loss of strength occurred to a certain extent after bleaching as hemicellulose, pectin, and lignin components were removed. Sodium hypochlorite was employed despite the shortcomings because of its low cost, availability, and easy handling compared to other bleaching agents.

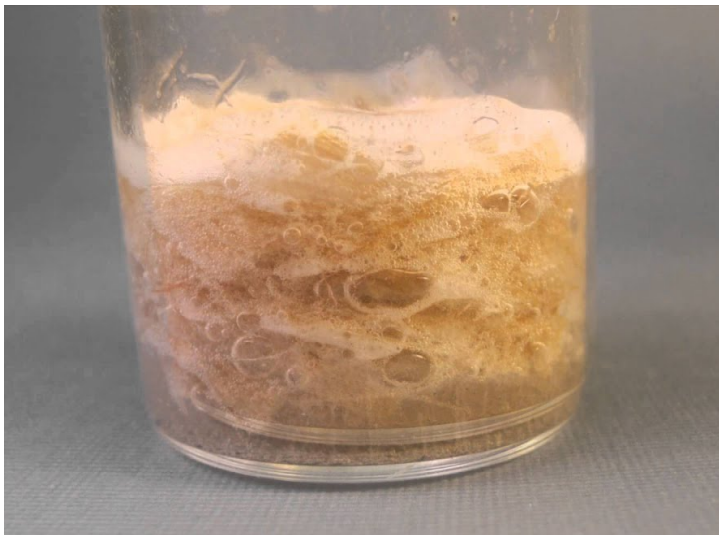


Figure 11: Bleaching of plant fiber

4.2.1.5 Grinding

The bleached fibers were ground to the desired length and with required surface roughness for proper bonding with an adsorbate. The locally available grinder was used and a speed of 78 rpm (revolution per minute) was selected.

4.2.1.6 Substrate molding

The slurry of the pulp of fibers was prepared. A mold with a wire screen was dipped into the solution and pulled out horizontally to create a sheet of the substrate as shown in Figure 12. Inverted petri dish was used in this work for scaled down molding. Depending upon the desired thickness the amount of slurry and pressure were varied. It was left in an open air for drying. The dry paper obtained was subjected to further studies regarding adsorption of essential oil.

Figure 12: Traditional preparation methods of paper sheets from pulp

4.3 Phytochemical methodology

The nature of solvents, the particle size and extraction conditions such as temperature, pressure, etc. governs the properties of extracted oil from plants.[84] The reflux and siphon principle to continuously extract the solid matter by pure solvent was used in Soxhlet extraction method (Figure 13). As it saves the solvent and is efficient, it is one of the most applied extraction procedures. Soxhlet extractor fitted with condenser and fine grounded plant material were placed in cellulose thimble in an extraction chamber. The solvent (*n*-hexane) at 65 °C for 3 hours for extraction of essential oil and solvent was removed from extracts using rotary vacuum evaporator.

Hydro-distillation uses water (steam) as an extraction solvent to recover volatile or polar components of plant materials (Figure 14). Hydro-distillation is preferred method as it does not require expensive solvent and essential oil used mostly for application on skin and it is gentle with sensitive skin. Hydro-distillation is carried out using a setup known as Clevenger apparatus. In the Clevenger apparatus, sample mixed water is boiled to evaporate volatile components. Two layers (aqueous and oil-rich fraction) are obtained and oil is further separated using separating funnel. The hydro distillate of samples were obtained using Clevenger apparatus using distilled water and 50 g of sample with 400 mL distilled water for 4 hours.

Figure 13. Extraction using Soxhlet extractor

Figure 14. Extraction using Clevenger apparatus

5. Conclusions and Future Insights

Computational

The computational investigation of three major phytochemicals from three medicinal plants of Nepal showed that Methyl-E-Cinnamate possesses better binding capacity with various receptors of SARS-CoV-2 than other candidates. It can thus be used as a volatile compound in face masks for fighting COVID-19. Inclusion of flexibility to the protein structure during docking provided more realistic nature of calculation and the details of non-covalent interactions at the active sites showed the necessity of the existence of hydrogen bonds or other electrostatic linkages for even better binding affinities. To determine the stability of the docked complexes and the possibility of inhibition of the protease, molecular dynamics simulation with times of at least 200 ns was required and would be pursued as future work along with additional phytochemicals from different chemical classes with higher molecular weight and druglikeness for better therapeutic values.

Phytochemical

The essential oil present in samples from different geographical location can vary due to environmental conditions. Therefore, further comparative analysis of samples from various parts of Nepal may provide comprehensive information on diversity of aromatic components and their principal components which can be responsible for curative properties. The use of isolated effective component or combination of components can enhance the effective formulation. The application of different fiber material and their essential oil holding capacity need further research. The adsorption properties of bamboo charcoal can be further enhanced by thermal or chemical activation.

Material

The fibrous substrates were prepared from eight different local plant sources and were used in holding the essential oils in an effective manner. The patch was fabricated from suitable fiber with better properties and the oil with the best therapeutic value as suggested by computational analysis was employed. Full scale kinetics study leading to the assessment of time of retention and release along with mechanical and thermal stability would be carried out as an extension to the project. This would ultimately yield a prototype material with the tailored properties. In this way, innovative product would eventually be designed and developed to combat COVID-19 and related infectious diseases.

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