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Network pharmacology approach to reveals therapeutic mechanism of traditional plants formulation used by Malaysia indigenous ethnics in coronaviruses infection

Fui Fui Lem [1,*], Dexter Jiunn Herng Lee [2], Fong Tyng Chee [3], Su Na Chin [4], Kai Min Lin [5,6] and Chee Wei Yew [2,*]

1 Clinical Research Centre, Hospital Queen Elizabeth, Ministry of Health Malaysia, 88586 Kota Kinabalu, Sabah, Malaysia; lemfuifui02@gmail.com; lemfuifui@moh.gov.my
2 Biotechnology Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia; dexlee8@gmail.com; cheewei.yew@ums.edu.my
3 Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Locked Bag No 3, 90509 Sandakan, Sabah, Malaysia; ftchee@ums.edu.my
4 Faculty of Science and Natural Resources Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia; chinsuna@ums.edu.my
5 Cambridge Institute for Medical Research, University of Cambridge, the Keith Peters Building, Cambridge Biomedical Campus, Hills Road, Cambridge CB20XY; kml53@cam.ac.uk
6 Department of Infectious Disease, Imperial College London, Exhibition Rd, South Kensington, London SW7 2BX, United Kingdom; kml53@cam.ac.uk
* Correspondence: lemfuifui@moh.gov.my (F.F. Lem); cheewei.yew@ums.edu.my (C.W. Yew)

Abstract: Network pharmacology analysis can act as a strategy to identify the pharmacological effect of plant-based bioactive compounds against coronavirus diseases. This study aimed to investigate the potential pharmacological mechanism of a local ethnomedicine (*Costus speciosus*, *Hibiscus rosa-sinensis* and *Phyllanthus niruri*) of Northern Borneo against coronaviruses known as CHP. Compounds in CHP were extracted from databases and screened for their oral bioavailability and drug-likeness before a compound-target network was built. Furthermore, the protein-protein interaction network and pathway enrichment were constructed and analyzed. A compound-target network consisting of 48 putative bioactive compounds targeting 587 candidate genes was identified. A total of 186 coronavirus-related genes were extracted and TP53, STAT3, HSP90AA1, STAT1 and EP300 were predicted to be the key targets. Notably, mapping of these target genes into the target-pathway network illustrated that functional enrichment was on viral infection and regulation of inflammation pathways. Urinatetralin is predicted, for the first time, as a bioactive compound that solely targets STAT3. The results from this study indicate that compounds present in CHP employ STAT3 and its connected pathways as the mechanism of action against coronaviruses. In conclusion, urinatetralin should be further investigated for its potential application against coronavirus infections.

Keywords: Network pharmacology; Malaysia indigenous ethnics; coronaviruses infection; therapeutic mechanism

1. Introduction

Over the last 20 years, different coronaviruses have emerged and caused outbreaks such as severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS) and the current novel coronavirus disease (COVID-19). These viruses, which are named as SARS-CoV-1, MERS-CoV and SARS-CoV-2, respectively, are believed to have zoonotic origins and are transmitted among the human populations (El-kafrawy et al., 2019; Wang et al., 2005; Zhou et al., 2020). In just a short outbreak of one year, the number of COVID-19 cases has exceeded 100 million, causing deaths of more than 2.2 million individuals by the end of January 2021. The (alveolar epithelial (AT1) cells) bronchial transient secretory cells on the lower respiratory tracts are infected (Lukassen et al., 2020), causing acute inflammation and subsequent pneumonia that could inflict death. Severe

pneumonia is induced by the uncontrolled overproduction of multiple inflammatory cytokines, which leads to lung injury and acute respiratory distress syndromes (Huang et al., 2020; Z. Xu et al., 2020; X. Yang et al., 2020). The majority of the deceased victims are older adults or those who were pre-diagnosed with diabetes, hypertension, rheumatic, cardiac and kidney diseases (Niu et al., 2020; Palmieri et al., 2020; Silva et al., 2020; Wang et al., 2020; Xiang et al., 2020; Zhou et al., 2020). An effective therapy is still needed; athough some antiviral drugs such as chloroquine (blocking the binding of virion to cell receptor), lopinavir–ritonavir (a HIV protease / CYP450 inhibitor) and remdesivir (an adenosine analogue blocking viral RNA replication) have been repurposed and intensively tested, their efficacy remains to be confirmed in clinical treatment (Cao et al., 2020; Colson et al., 2020; C. J. Gordon et al., 2020; Ko et al., 2020). As such, there is an urgency to find potential drug candidates that effectively act against coronavirus diseases.

Although the genome of SARS-CoV-2 only shares 79.5% and 50% amino acid sequence identity with SARS-CoV-1 and MERS-CoV, respectively (Lu et al., 2020), they have a common genomic structure and functional proteins; for example, those that mediate the entry (Spike glycoprotein, S), replication (RNA-dependent RNA polymerase, RdRp) and assembly (envelope protein, E; membrane protein, M; nucleocapsid protein, N) of the viruses in the host cells (Lu et al., 2020; Zhou et al., 2020; Zumla et al., 2016). However, the key differences at the receptor-binding domain of the Spike (S) protein between SARS-CoV-1 and SARS-CoV-2, which form a complex with the host cell receptor, angiotensin-converting enzyme 2 (ACE2), could have attributed to its higher potential of transmissibility in comparison with other coronaviruses (Hu et al., 2021; X. Xu et al., 2020). Nevertheless, subsequent network analyses of the virus-host protein-protein interactome have revealed shared pan-coronavirus viral-disease molecular mechanisms on transmission and replication (D. E. Gordon et al., 2020a). These interacting proteins could be new therapeutic targets for repurposed drugs or discovery of new bioactive molecules (Chassey et al., 2014; D. E. Gordon et al., 2020b).

The spread of the SARS-CoV-2 virus could be stopped by reaching herd immunity after the majority of human beings are vaccinated. However, the emergence of coronaviruses over the last 20 years, which are notorious for inducing acute respiratory distress syndrome (ARDS), should be taken as an alarming sign as we know that these viruses are spillovers from reservoirs or intermediate hosts that are encountered through human activities. As vaccines are specifically designed to target prevalent strains of viruses, spontaneous mutations could render the vaccines ineffective. Although the covid-19 vaccines have become readily available in just a year time, which is the fastest in the human history, sufficient distribution of the vaccines and completion of the subsequent vaccination programs may take another year to be completed. Taking covid-19 as an example, millions of lives could be claimed during this period. As such, natural products with polypharmacological actions should be investigated and mined as a preparedness strategy for the next emerging coronavirus.

Medicinal plants serve as a huge reservoir of bioactive molecules, contributing to almost 25% of modern drugs (Orhan, 2012). A number of plant-based traditional Chinese medicines such as Hanshiyi and Qinfei Paidu decoctions have already been clinically tested to reduce the transition of mild symptoms to severe ones in COVID-19 patients, which has been attributed to their anti-inflammatory protection effect (Tian et al., 2020; Xin et al., 2020). Considering the rich biodiversity of plants found in the tropical rainforest of northern Borneo, natural products extracted from plant-based ethnomedicines that are traditionally used by a local indigenous community, Kadazan-Dusun, could be explored for their medicinal potentials. For example, the root of Alstonia scholaris, which has been used for treating fever by the local community (Ahmad and Holdsworth, 2003), has been found to inhibit γ coronavirus through the compounds alstotides extracted from the plants (Nguyen et al., 2015). In this study, an ethnomedicine which is a cooked tea made up of three local plants known as Sibhu-sibhu (Costus speciosus Sm.), Tongkuango (Hibiscus rosa-sinensis L.) and Nipon-nipon (Phyllanthus niruri L.) (subsequently

abbreviated as CHP), was investigated for its antiviral effect. It has traditionally been used to relieve flu-like symptoms such as fever, cough and shortness of breath. Furthermore, the use of the individual plants (as summarized in Table 1) for the relief of asthma, hypertension or even malaria infection (Ahmad and Holdsworth, 2003; Kodoh et al., 2017) has been documented in other indigenous communities; however, there is a lack of scientific evidence for their therapeutic effect. Furthermore, previous screening of their biochemical properties demonstrated that the individual plant extracts of each possess anti-inflammatory (IH, 1966), anti-viral (Álvarez et al., 2012; Forero et al., 2008; Naik and Juvekar, 2003; Venkateswaran et al., 1987; Wahyuni et al., 2019; Xiang et al., 2008) and anti-microbial effects (Saraf, 2010). As the targeted symptoms are similar to those associated with coronavirus infection and comorbidities, the synergistic effects of the bioactive compounds found in the CHP ethnomedicine deems further analysis through a network pharmacology approach.

In line with this, the aim of this study was to, first, predict the bioactive molecules from the known chemical compounds present in each plant. Second, the target proteins from both coronaviruses or human hosts, were determined from databases and published papers. Next, the compound-target network was constructed and then mapped to the protein–protein interaction network of a human host and coronaviruses, for the identification of molecular pathways modulated by the bioactive compounds. The approach of network pharmacology can be used to predict the synergistic effects of the bioactive compounds (Fang et al., 2018) found in CHP on multiple cellular protein targets that mediate numerous biological pathways involving viral infection and host immune defense. As such, the mechanism of actions of this ethnomedicine against coronavirus infection was described.

Table 1. Traditional use of plants part in treating viral-infection related clinical symptom by indigenous ethnic people in Northern Borneo.

Plant name	Traditional usage	Treatment	References
(Costus speciosus (J. Koenig) Sm.)	Peeled stems are steeped in hot water as drinking water	Asthma Influenza	(Ahmad and Holdsworth, 2003)
	Rhizomes are boiled as tea	cough	(Ahmad et al., 1994)
	Root	Fever Cough	(Institute Medical Research, 2018)
Tongkuango (*Hibiscus rosa-sinensis* L.)	Sap from shoots is drunk as water	Asthma	(Ahmad and Holdsworth, 2003)
	Flowers are boiled and packed as patch	Yellow fever	(Kodoh et al., 2017)
Nipon-nipon (*Phyllanthus niruri* L.)	Plants are pounded into a paste and squeezed for the juice	Fever Malaria Hypertension	(Ahmad and Holdsworth, 2003)

2. Results

2.1. Putative bioactive compounds and targeted genes

A total of 239 compounds were collected from C (81), H (109) and P (48) (supplementary Table 1). After filtering with the ADME criteria, a total of 49 compounds constituting 18 (C), 17 (H) and 14 (P) compounds, respectively, were considered as bioactive with drug properties, as summarized in Table 2 according to the OB and DL criteria set in section 4.2.

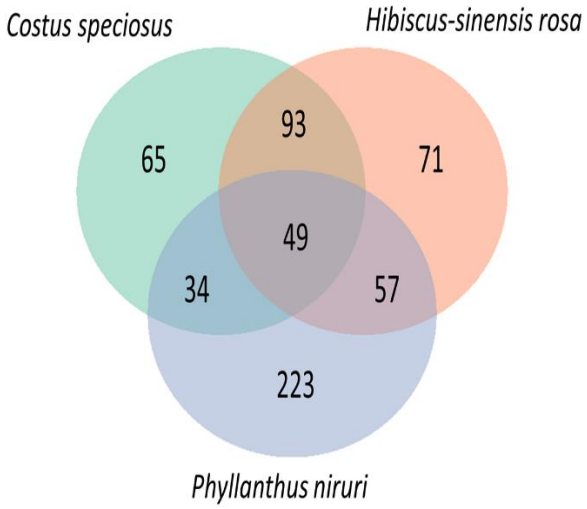
Table 2. The list of putative bioactive compounds found in the CHP ethnomedicine.

Plant species	Molecule	Formula
Costus speciosus (J.Koenig) Sm (C)	1,8-Cineole	C10H18O
	3-(4-hydroxyphenyl)-2E propenoate	C9H8O3
	Borneol	C10H18O
	Bornyl acetate	C12H20O2
	Camphor	C10H16O
	Caryophyllene oxide	C15H24O
	Costunolide	C15H20O2
	Coumarin	C9H6O2
	Eremanthin	C15H18O2
	Gramine	C11H14N2
	Humulene epoxide II	C15H24O
	lauric acid	C12H24O2
	Linalool	C10H18O
	Terpinen-4-ol	C10H18O
	vanillin	C8H8O3
	zerumbone	C15H22O
	α-Terpineol	C10H18O
	β -Eudesmol	C15H26O
Hibiscus-sinensis rosa L. (H)	10-oxo methyl ester	C12H22O3
	2, 2, 4-trimethyl 3- pentanone	C8H16O
	2-cyclopentylethanol	C7H14O
	4- trifluoroacetoxyoctane	C10H17F3O2
	7-Formylbicyclo(4.1.0) heptanes	C8H12O
	8-Nonynoic	C9H14O2
	9-decynoic acids	C10H16O2
	amylnitrite	C5H11NO2
	anthocyanin	C15H11O
	dec-9-ynoic acid methyl ester	C11H18O2
	decanoic acid	C10H20O2
	lauric acid	C12H24O2
	niacin	C6H5NO2
	non-8-ynoic acid methyl ester	C10H16O2
	nonanoic acid	C9H18O2
	octanoic acid	C8H16O2
	undecanoic acid	C11H22O2
Phyllanthus niruri L. (P)	Cubebin dimethyl ether	C22H26O6
	Estradiol	C18H24O2
	hinokinin	C20H18O6
	hypophyllanthin	C24H30O7
	isolintetralin	C23H28O6

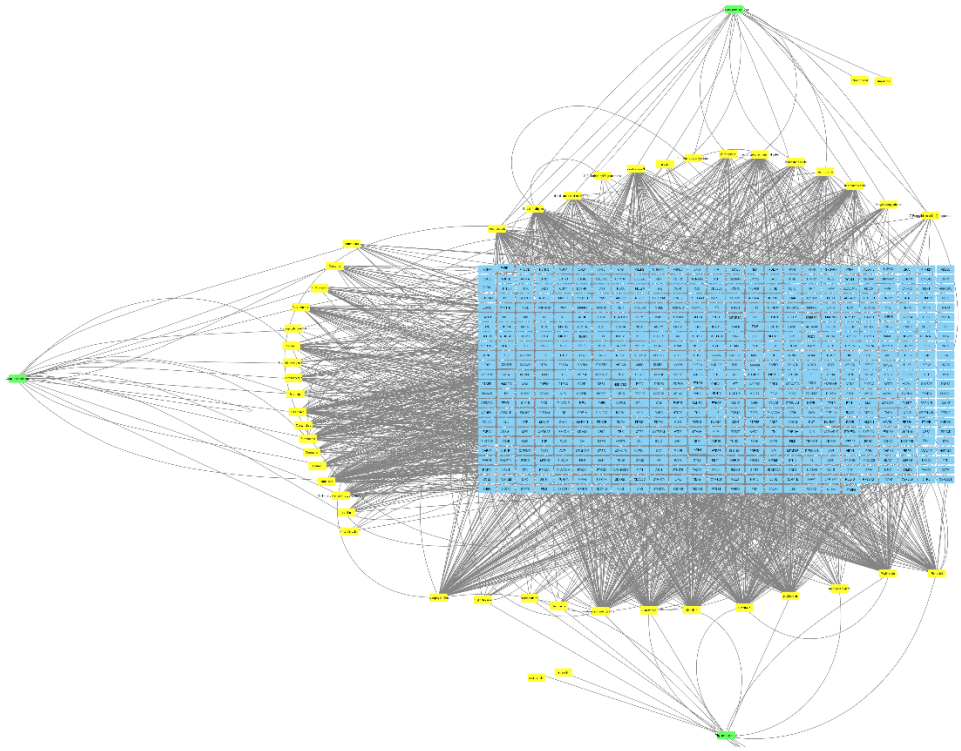
lintetralin	C23H28O6
methyl-salicylate	C8H8O3
Nirtetralin	C24H30O7
Norsecurinine	C12H13NO2
Phyllnirurin	C20H22O5
phyllochrysine	C13H15NO2
phyltetralin	C24H32O6
Securinine	C13H15NO2
Urinatetralin	C22H24O6

2.2. CHP compound-target genes network analysis

A total of 588 non-redundant targets contributing C (241), H (270) and P (363) were predicted (Figure 1a). Four compounds, octanoic acid and amylnitrile from H and isolintetralin and lintetralin from P, did not fulfil the prediction criteria. Only the predicted targets with probability >0 were included in the compound-target network (Figure 1b) for high confidence of the predicted target from the databases.



(**a**)



(**b**)

Figure 1. Analysis of compound-target network constructed based on 49 CHP bioactive compounds and 588 putative target genes. (a) The Venn diagram shows common and unique number of targets by compounds from different plants. (b) The illustration of a compound-target network.

2.3 Mapping of the coronavirus-related genes to protein-protein interaction network of the target genes

A total of 186 coronavirus-related genes were extracted from a public database. These genes were then compiled with the CHP compound targets. Predicted targets with the highest confidence value of 0.98 were used to construct the compound-target protein-protein interaction network (PPI). The resulting PPI was made up of 721 nodes linked by 421 edges, with an average node degree of 1.17 and average local clustering coefficient of 0.228. Notably, MCL clustering of all nodes grouped 25 clusters in the PPI network (Figure 2). To better understand the mechanism of CHP in coronavirus, PPI was constructed and their topological properties such as betweenness centrality (BC) and connectivity degree were analyzed, the fundamental measures nodes in network theory (Barabási et al., 2011), using Network Analyzer plugin in Cytoscape Version 3.8. Analyses of the node degree and betweenness centrality (data shown in Supplementary Table 2) revealed 20 hub nodes and 20 bottleneck nodes, and five key genes were identified from the hub/bottleneck nodes, as shown in Table 3. Analyses of PPI predicted that TP53 (31) and STAT3 (24) are the hub nodes attributed to the largest degree of connectivity with other genes (Supplementary Table 2). In addition, these two genes were also predicted to be key proteins (i.e., with high degree of BC) together with HSP90AA1, STAT1 and EP300 as the key proteins that we identified from the PPI, which are the pivotal genes that correspond to the pathogenesis of the coronavirus infection. In the meantime, bottleneck nodes, which are known to lead to network "traffic" chaos, were also identified: F3, CNR1, DRD2, SMO, SQLE and F2. We also found that TP53 and STAT3 were also defined as bottleneck nodes since hub and bottleneck nodes show a tendency to be indistinguishable (Charitou et al., 2016). In this study, we focused on proteins with large degree and high BC; i.e., TP53, STAT3, HSP90AA1, STAT1 and EP300 as the key proteins.

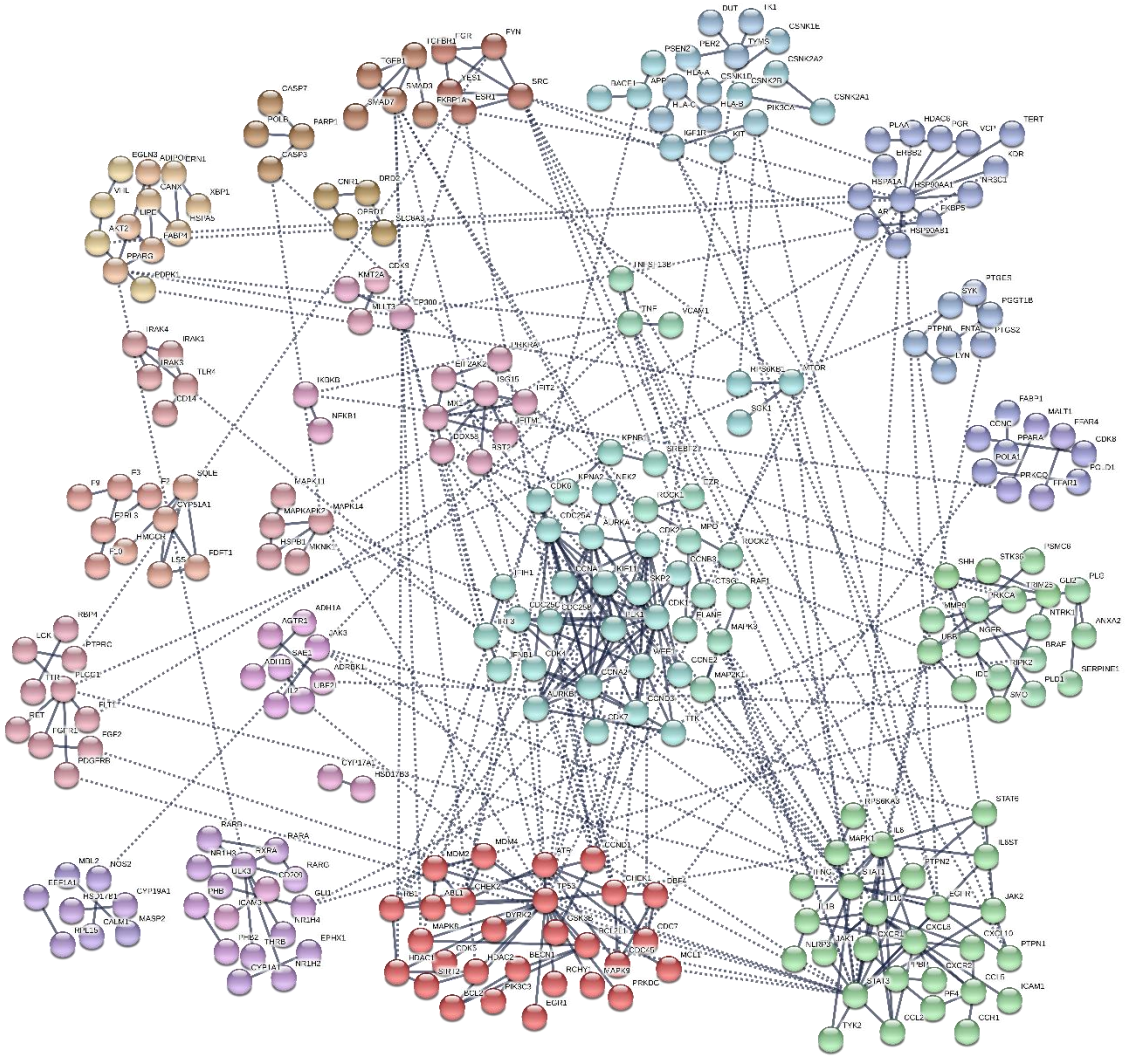


Figure 2. Constructed protein–protein interaction network from the compiled predicted targets of CHP and coronavirus-related genes. MCL clustering resulted in 25 clusters with 2 main subclusters containing hub nodes of TP53 (red) and STAT3 (green)

Table 3. Hub and bottleneck nodes in PPIN.

Hub nodes	TP53, STAT3, CDK1, CCNA2, HSP90AA1, CDK2, STAT1, CDC25A, SRC, IL6, CCL8, CCND1, IL10, TNF, EP300, PLK1, AURKA, CHEK1, ISG15, RXRA
Bottleneck nodes	HLA-A, KPNB1, F3, CNR1, DRD2, SMO, SQLE, F2, TP53, GLI1, STAT3, GRK2, HSP90AA1, MAPK14, EP300, STAT1, GLI2, TNF, PPARG, IRF3
Key genes	TP53, STAT3, HSP90AA1, STAT1, EP300

2.4 Mechanism-of-action of anti-coronavirus indications mediated by bioactive compounds

Gene ontology (GO) analysis revealed that most of the existing potential targets were enriched in the cellular response to chemical stimulus, cellular response to organic substances, regulation of biological quality, response to oxygen-containing compound and positive regulation of biological process (Figure 3). GO analyses showed that among the top 20 enriched virus-related biological processes (Figure 4a) were regulation of defense response (2.60x10-42), regulation of T cell activation (1.38x10-17), response to virus

(4.60x10-13), defense response to virus (7.44x10-07) and regulation of interleukin-6 production (5.83x10-06). Cell, cytoplasm, membrane and intracellular components were the cellular components that most of the target genes involved (Figure 3b). Notably, Kaposi's sarcoma-associated herpesvirus infection (1.90x10-26), influenza A (1.27x10-25), hepatitis B (5.55x10-25) and Epstein-Barr virus infection (3.79x10-24) were identified among the top 20 KEGG pathways, as demonstrated in Figure 3c. As shown in Figure 3d, molecular functions that are mostly involved by CHP are catalytic activity and drug binding with p-value of 7.31x10-81 and 2.42x10-77, respectively.

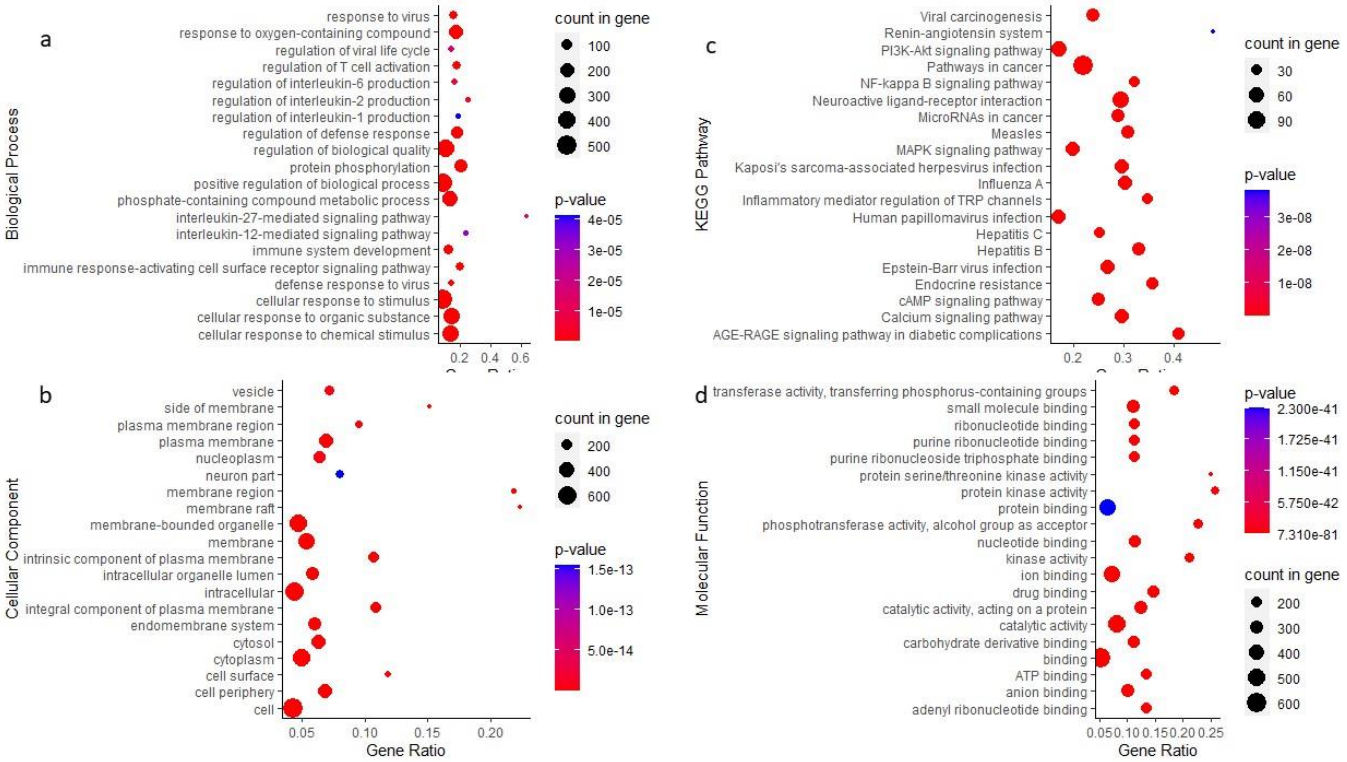


Figure 3. Enrichment analysis of CHP and coronaviruses target gene in accordance with (**a**) biological process, (**b**) cellular component, (**c**) KEGG pathway and (**d**) molecular function.

3. Discussion

To the best of our knowledge, this is the first study to postulate the mechanism of action of CHP, which has been traditionally used by the indigenous Kadazan-Dusun people of Borneo, against coronavirus infection using a network pharmacology approach. Analysis of pathway enrichment showed that majority of the targeted proteins play roles in membrane/cell surface, membrane binding, signal transduction, phosphorylation, kinases activities, viral response, regulation of viral cell cycle, cytokine signaling, regulation of inflammation and activation of T cells (Figure 3). Notably, these pathways illustrate general mechanisms of viral pathogenesis and induction of an immunity response caused by coronaviruses (D. E. Gordon et al., 2020a). This indicates that multiple bioactive compounds in the CHP ethnomedicine could act in synergy to mediate cellular response against infection of coronaviruses and subsequent induction of immune defense mechanisms. Nevertheless, it should be noted that different medicinal plants could employ a different mechanism of action against coronavirus. For instance, Qingfei paidu decoction was proposed to mediate anti-inflammation induced by Covid-19 via the Toll-like (TLR) signaling pathway (R. Yang et al., 2020) and Shuang Huang Lian Kou fu ye mitigate the Covid-19 through inhibiting the angiotensin-converting enzyme (ACE) (Lem et al., 2021).

The results from this study suggest that CHP employs STAT3 and its connected pathways as the mechanism of action against coronaviruses. According to PPI, the STAT3

protein was predicted to be a hub gene forming a subcluster and connected directly to MAPK1, TP53, IL6, HSP90AA1 and indirectly to EP300 and STAT1, which are also putative key genes as inferred by the network analysis. STAT3 has been found to be versatile and plays a central role in response to viral infection, immunity such as chemokine signaling pathway, and neutrophil trafficking during acute inflammation (Chang et al., 2018; Fielding et al., 2008; Kim et al., 2019; Shamir et al., 2020). Coronaviruses such as SARS-CoV cause SARS infection by mediating viral replication in target cells and immune response. It activates the p38 MAPK pathway, which induces STAT3 dephosphorylation at Tyr705, which eventually promotes SARS-CoV replication (Mizutani et al., 2004). In addition, the IL-6/STAT3 pathway promotes inflammation. IL-6 is a classic pro-inflammatory cytokine that signals through STAT3 as part of the acute phase response that aims to attract macrophages that destroy the infected cells and clearance of virus particles (Choy and Rose-John, 2017; Suarez et al., 2018). Acute respiratory distress syndrome (ARDS), which is the inflammatory injury to the alveola-capillary membrane that leads to acute pneumonia and respiratory insufficiency, is regarded as the hallmark of immune-mediated clinical consequence after infection by SARS-CoV-2 (nCoV-2019) (Huang et al., 2020; Z. Xu et al., 2020). ARDS is the ultimate result of a cytokine storm (Coperchini et al., 2020). As such, the mechanism of action acting against STAT3 could be further explored as a potential drug target for inflammation (Hu et al., 2019).

Furthermore, mapping of the compound-target proteins to coronavirus-related genes indicated that out of the key genes above only STAT3, MAPK1 and HSP90AA1 were predicted to be targeted by at least one putative bioactive compound (Supplementary Table 4). It is notable that STAT3 is only targeted by Urinatetraline, which was only found in Phyllanthus niruri. HSP90AA1 was targeted by 10-oxo methyl ester from Hibiscus-sinensis rosa, and hinokinin and hypophyllantin from Phyllantus niruri; MAPK1 was targeted by lauric acid from Hibiscus-sinensis rosa and Costus speciosus, and phyllinirurin from Phyllanthus niruri. A special note should be made about urinatetralin, a unique lignan of phenylpropanoid compound first discovered and purified from an ethanol/hexane extract of Phyllanthus urinaria root (Chang et al., 2003) and then from a methanol extract of cell suspension cultures developed from the leave callus of Phyllanthus niruri (Batterman et al., 2006). The pure compound of urinatetralin has not been purified for in vitro or in vivo testing of its pharmacological effect since then (Lee et al., 2016; Li et al., 2009), even though the crude methanolic extract from the leaves was tested to possess anti-inflammatory effects on inflammation-induced laboratory rats (Mostofa et al., 2017). In this study, urinatetralin was first predicted to be a bioactive compound that passed the ADME criteria. More importantly, it targets STAT3, which is the hub gene of the PPI network made up of all predicted targets and coronavirus-related genes. This is in agreement with Matsuyama et al. (2020), who proposed that SARS-CoV-2 mediates the dysregulation of the STAT3 signaling networks, which result in the pervasive pathological features of COVID-19 (Matsuyama et al., 2020).

However, this result should be interpreted with caution, as the currently known clinical outcomes of Covid-19 mediated by these pathways remains hypothetical and extensively debated. For instance, a special note was made on the renin-angiotensin system (RAS), which had the highest gene ratio obtained from KEGG pathway enrichment analysis (Figure 4b). The RAS has two key enzymes, ACE1 and ACE2, which control the balance of the angiotensin family. It is well-known that ACE2 is used for viral cell entry; thus it can be intuitively conceived that the greater the amount of ACE2 in the cell membrane, the greater the chance of viral entry into multiple cell types expressing ACE2, leading to ARDS and multi-organ failure in severe cases. In contrast, children who naturally have higher ACE2 as compared with elders, usually have very mild symptoms if infected (Dong et al., 2020). A hypothesis on the RAS imbalance was recently proposed (Lanza et al., 2020), trying to answer the reason why ARDS are significantly higher in elders with diabetes, hypertension and cardiovascular diseases as comorbidities. These patients are usually treated with ACE inhibitors (which block the action of ACE1) or angiotensin

receptor blockers (ARB, which block the action of angiotensin II) of the RAS pathway, resulting in decreased ACE1 but upregulation of ACE2 (Furuhashi et al., 2015). An in vivo study showed that the Spike protein led to down-regulation of ACE2 and more severe lung injury in mice, which could be attenuated by administration of ARB (Imai et al., 2005; Kuba et al., 2005). It was hypothesized that excessive ACE2 may competitively bind with SARS-CoV-2 Spike protein and supplement cellular ACE2 activity, which negatively regulates the RAS to protect the lung from injury (Kuster et al., 2020). However, this plausible protective therapeutics against advanced stage COVID-19 remains clinically unproven until tried (Aronson and Ferner, 2020; Gurwitz, 2020). In this study, instead of ACE2, the other component genes of the RAS pathway, i.e., CMA1, CPA3, CTST, MME, PRCP and REN (Nehme et al., 2019), are predicted to be targeted by CHP bioactive compounds (Supplementary Table 4). In line with the current debated hypotheses above, this suggests that targeting the component genes could be helpful to regulate the level of ACE2 in the infected cells and be extrapolated to be protective against ARDS.

Although CHP could be effective against coronaviruses, there are a number of limitations that should be considered. First, the ethnomedicine cocktail is made up of multiple natural products in the form of a crude extract. All the currently identified compounds only represent a subset of the total compounds in all three plants. The outcome could be attributed solely or in combination with other new compounds that have not been previously identified. Second, the ethnomedicine has synonymous concept of combination therapy, i.e, the use of multiple drugs to improve clinical outcomes, but systematic high-throughput testing of its combination and dosage could not be conducted. It is used by intuition and experience gained from traditional knowledge. Third, the current findings are based upon computational prediction, but not on experimental verified drug-target interactions, as compared with drug repurposing. An intuitive and straightforward way to identify new targets for a drug is to compare the candidate proteins with those existing targets of that drug. Different results may be obtained depending from which perspective the comparison was made. Fourth, the elusive mechanism of viral infection remains to be discovered. This affects the comprehensiveness of the prediction since only a known, limited number of protein associated to host–virus interactome (inclusive of defense, regulation and other cellular pathways) were extracted from recent published studies.

4. Materials and Methods

4.1 Prediction of bioactive compounds and target genes

Costus speciosus (J.Koenig) Sm., *Hibiscus rosa-sinensis* L. and *Phyllanthus niruri* L., (abbreviated as CHP) have been verified for their name from http://www.theplantlist.org/. Known chemical constituents of CHP were extracted from three databases, which were Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/phytochem/search), Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://www.tcmspw.com/tcmsp.php) and Bioinformatic Analysis Tool for Molecular Mechanism of Traditional Chinese Medicine (BATMAN-TCM, http://bionet.ncpsb.org/batman-tcm) (Liu et al., 2016; Ru et al., 2014; U.S. Department of Agriculture, 2016). Updated findings of each plant species were also referred (Al-Snafi, 2018; Bagalkotkar et al., 2006; El-far AH, Shaheen, HM, Alsenosy, AW, El-Sayed YS, Al Jaouni SK, Mousa, 2018; Jadhav et al., 2009; Kamruzzaman and Hoq, 2016; Kumar et al., 2018b, 2018a; Missoum, 2018; Mohamad et al., 2018; Narendra et al., 2012; Pawar and Pawar, 2014; Qiao et al., 2002; Rani et al., 2012; Salem et al., 2014; Thabit, 2018; Thambi and Shafi, 2015; Waisundara et al., 2015) and compiled into a list of compounds (Supplementary Table 1). Compounds that do not possess the canonical simplified molecular-input line-entry system (SMILES) in PubChem were removed.

4.2 Screening of chemical constituents according to oral bioavailability (OB) and druglikeness (DL) and target prediction of filtered bioactive compounds

Each compound was screened to measure its oral bioavailability (OB) and druglikeness (DL) in accordance with the absorption, distribution, metabolism and excretion (ADME) properties. The OB and DL properties were calculated using SwissADME (http://www.swissadme.ch/) (Daina et al., 2017). Additional criteria were set for the compounds to be high in gastrointestinal (GI) absorption, able to cross the blood-brain barrier (BBB), and not violate both Lipinski's rule of five (Lipinski, 2004) and Veber's rule (Veber et al., 2002). Target prediction for each bioactive compound was conducted with the SwissTargetPrediction database (http://www.swisstargetprediction.ch/), depending on chemical similarities and limited to Homo sapiens and probability > 0 to possess high confidence level of the predicted target through extracting extensive information of known experimental bioactivity (Gfeller et al., 2014). Duplicates were removed and the name of each gene was standardized in accordance with UniProt database (http://www.uniprot.org/).

4.3 Construction of compound-target network

The putative bioactive compounds and predicted target genes retrieved from the databases were inputted into Cytoscape version 3.8 (Shannon et al., 2003) to construct the compound-target network for better visualization. The pharmacology of CHP was characterized using the multi-compound and multi-target network. In addition, the unique and shared target genes of each individual plant was analyzed and visualized with a Venn diagram.

4.4 Acquisition of coronavirus-related genes

Coronavirus-related genes that are known to be associated with coronavirus diseases were searched and screened to identify biological attributes relevant to the pathogenesis of coronavirus viral infections. The coronaviruses included in the analyses were alphacoronavirus, betacoronavirus, gammacoronavirus, severe acute respiratory syndrome-related (SARS) coronavirus, Middle East respiratory syndrome-related (MERS) coronavirus, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) according to the search keywords shown in Supplementary Table 3. Two databases, Online Mendelian Inheritance in Man (OMIM, https://omim.org/) and National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/gene?cmd=Retrieve) (Agarwala et al., 2016; Amberger et al., 2019) were used to search for coronavirus-related genes using these keywords.

4.5 Identification of hub and key target genes via protein-protein interaction network

All compound-target and coronavirus-related genes were compiled and duplicates were removed. This compiled set of genes was inputted into STRING 11.0 (https://string-db.org/) database (Szklarczyk et al., 2019) to obtain the interactions information among the proteins. A confidence score of 0.98 was selected for the highest confidence of the interaction and clustered with an inflation value of 1.8 using Markov Clustering (MCL) before it was imported to Cytoscape Version 3.8 (Shannon et al., 2003) to visualize the graphical network. MCL clustering was used to group highly interconnected nodes, revealing interplay between myriad pathways. The value of 'degree', which is the number of links to each node (protein) was referred. The application 'NetworkAnalyzer' was then used to calculate the node 'degree' and 'betweenness centrality' (BC), which serve as the key topological parameters for the PPI network. Key genes were identified from the hub and bottleneck nodes with high node degree and BC value.

4.5 Identification of hub and key target genes via protein-protein interaction network

To investigate the pharmacological potential of CHP bioactive compounds, the underlying mechanism of action on biological pathways of the candidate targets were referred. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG)

pathway enrichment analysis were performed by STRING 11.0 (Kanehisa et al., 2019; Szklarczyk et al., 2019; The Gene Ontology Consortium, 2019). Only statistically significant (p-value < 0.05) biological process or pathways were subsequently analyzed with R version 3.6.3 (RStudio Team, 2020).

5. Conclusions

Analyses of network pharmacology of CHP ethnomedicine showed that its bioactive compounds should have plausible synergy in regulating inflammation via STAT3-mediated pathways, upon infection of human subjects by coronaviruses. In this study, urinatetralin was predicted, for the first time, to be a bioactive compound that passes the ADME criteria. More importantly, it targets STAT3, which is the hub gene of the PPI network made up of all predicted targets and coronavirus-related genes. Because STAT3 is well-known for its versatility in mediating inflammation and viral infection, further analyses and evidence-based experiments should be conducted.

Supplementary Materials: Figure S1: title, Table S1: Oral Bioavailability and drug likeness of listed compounds for CHP, Table S2: Network properties of target gene, Table S3: Search keywords for coronaviruses targets in OMIM and NCBI databases, Table S4: List of genes targeted by compounds in CHP.

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Data Availability Statement: The data analyzed in this study is subject to the following licenses/restrictions: No restrictions. Requests to access these datasets should be directed to FFL, lemfuifui@moh.gov.my.

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