The role of andrographolide and its derivative in COVID-19 associated proteins and immune system

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Abstract

Aim: In view of the strong immunomodulatory and antiviral activity of andrographolide and its derivative, the present study aimed to investigate the binding affinities of andrographolide and its derivative 14-deoxy-11,12-didehydroandrographolide with 3 major targets of COVID-19 i.e. 3CLpro, PLpro and spike protein followed by their gene-set enrichment analysis with special reference to immune modulation.

Materials and methods: SMILES of the compounds were retrieved from DigePred database and the proteins identified were queried in STRING to evaluate the protein-protein interaction and modulated pathways were identified concerning the KEGG database. Drug-likeness and ADMET profiles were evaluated using MolSoft and admet SAR 2.0, respectively. Molecular docking was carried using autodock 4.0.

Results: Andrographolide and 14-Deoxy-11,12-didehydroandrographolide were predicted to have a high binding affinity with papain-like protease i.e. -6.7 kcal/mol and -6.5 kcal/mol, respectively while they interact with equal binding energies with 3clpro (-6.8 kcal/mol) and spike protein (-6.9 kcal/mol). Network pharmacology analysis revealed that both compounds modulated the immune system through the regulation of chemokine signaling pathway, Rap1 signaling pathway, Cytokine-cytokine receptor interaction, MAPK signaling pathway, NF-kappa B signaling pathway, Rassignaling pathway, p53 signaling pathway, HIF-1 signaling pathway, and Natural killer cell-mediated cytotoxicity. Although the 14-deoxy-11,12-didehydroandrographolide scored higher drug-likeness character, it showed less potency to interaction with targeted proteins of COVID-19.

Conclusion: The study suggests the strong interaction of the andrographolide and its derivative 14-deoxy-11,12-didehydroandrographolide against target proteins associated with COVID-19. Further, network pharmacology analysis elucidated the different pathways of immunomodulation. However, clinical research should be conducted to confirm the current findings.

Introduction

In December 2019, severe acute respiratory syndrome caused by novel severe acute respiratory syndrome novel coronavirus 2 (SARS-nCoV-2) (Zhu et al., 2020) emerged as a global pandemic from the Wuhan city, Hubei province, China. WHO designated this nSARS-CoV-2 infection as COVID-19. The nSARS-CoV-2 s a highly contagious virus that can be transmitted from person to person (Anonymous, 2020) leading to community transmission. COVID-19 has become a major global threat by influencing around 212 countries with almost half million death throughout the globe (Sinha et al., 2020a). It has majorly affected the subjects with comorbidity and low immunity who are suffering from infectious and non-infectious diseases (Opitz et al. 2010). Patients with COVID-19, especially those with severe pneumonia, showed substantially lower lymphocyte counts and severely ill patients exhibited reduction in CD4+ T cells, CD8+ T cells, and natural killer cells. (Huang et al., 2020; Wang et al., 2020) The higher plasma concentrations of a number of inflammatory cytokines such as IL-6 and tumor necrosis factor (TNF) were
observed in COVID-19 patients (Wan et al., 2020). The pathological findings in patients with COVID-19 showed that immune-mediated lung injury was involved in acute respiratory distress syndrome (ARDS) (Xu et al., 2020). These evidences suggested immune imbalance in COVID-19 and it was contemplated that the immune system modulation can provide some prophylaxis and promising benefit against COVID-19 (Zhang et al., 2020). Hence, there is need to utilize the concept to identify the new therapeutic agent with immunomodulatory action as well as anti-viral property against the COVID-19.

The effectiveness of treatment based on traditional medicinal plants has been reported during the 2003 SARS (Wen et al., 2011; Chen et al., 2008; Lin et al., 2008; Ryu et al., 2010). Therefore, the scientific community has already started studies on medicinal plants, based on their history and traditional uses, as plausible leads in the treatment of Covid-19 (Chikhale et al., 2020; Sinha et al., 2020a,b).

For thousands of years, medicinal plants have been playing important role in the management of multiple infectious and non-infectious diseases (Mahady 2005; Sofowora et al 2013; Bahmani et al 2015). Among them, *Andrographis paniculata* (Family: Acanthaceae) also called known as King of bitters and Indian Echinacea, reserves its importance in the management of various infectious and non-infectious diseases (Wintachai et al., 2015; Pongtuluran and Rofaani 2015; Zhang and Tan 2000; Mopuri et al 2015). Further, it has been well studied for its potency as a modulator of the immune system (Pongtuluran and Rofaani 2015; Puri et al., 1993). Among the multiple phytoconstituents present in *Andrographis paniculata*, andrographolide (Mishra et al 2011) is a major bioactive which possesses its beneficial effect in multiple pathogenic conditions including immune booster role (Wang et al 2010). Further, two important databases i.e. ChEBI and PCIDB also record andrographolide as chief bioactive from *Andrographis paniculata*. Andrographolide and its derivatives exhibited strong immunomodulatory action (Churiyah et al., 2015; Puri et al., 1993) and is reported to have broad spectrum antiviral properties (Gupta et al., 2017). It was found effective against viral infections like dengue (Edwin et al., 2016), swine flu (Seniya et al 2014), hepatitis C (Lee et al., 2014), chickengunia (Wintachai et al., 2015), influenza (Chen et al., 2009), Epstein-Barr virus (EBV) (Lin et al. 2008) and herpes simplex virus 1 (HSV-1) (Wiart et al. 2005) in previous experimental studies.

Hence, the present study aimed to investigate the prospective potential andrographolide as a potent anti-viral agent by targeting three proteins of COVID-19 i.e. 3clpro, PLpro, and spike protein. Further, the study also evaluated the probable pathways to be regulated in enhancing the immune system. Since, during the mining of the andrographolide derivatives, we identified 14-deoxy-11,12-didehydroandrographolide as its derivative, hence the study also evaluated its potency for the anti-viral property against COVID-19 and the probably modulated pathways.

**Materials And Methods**

**Prediction of targets**

SMILES of 14-deoxy-11,12-didehydroandrographolide and andrographolidewas retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and was queried for protein-based prediction in
DIGEP-Pred (Lagunin et al 2013) at a probable activity (Pa) > Probable inactivity (Pi).

**Enrichment analysis**

The list of up-and down-regulated proteins was queried in the STRING database (Szklarczyk et al 2017). The biological process, cell component, and molecular function were recorded. The modulated protein and their associated pathways were identified using the KEGG pathway database. The interaction between the compounds, their targets, and pathways was constructed using Cytoscape which was further analyzed using edge count.

**In silico molecular docking**

3D structure of 14-deoxy-11,12-didehydroandrographolide and andrographolide was retrieved from the PubChem database in .sdf format and converted into .pdb format using Discovery studio 2019. The ligand of each molecule was minimized using mmff94 forcefield and converted into .pdbqt format. Targets 3clpro (PDB: 6LU7), and PLpro (PDB: 4M0W) were retrieved from the RCSB database (https://www.rcsb.org/) which were in complexed with water molecules and hetero atoms; removed using discovery studio 2019 and saved in .pdb format. Spike protein of coronavirus was homology modeled target by using accession number AVP78042.1 as query sequence and PDB: 6VSB as a template using SWISS-MODEL (Schwede et al., 2003). Docking was carried using autodock4 (Morris et al., 2009). After docking ten different confirmations of ligand were obtained in which ligand possessing minimum binding energy was chosen to visualize the ligand-protein interaction using discovery studio 2019.

**Calculation of drug-likeness and ADMET**

Drug-likeness of the compound was calculated based on the Rule of five using Molsoft (https://molsoft.com/mprop/) by querying the SMILES of compounds. Further, absorption, distribution, metabolism, and excretion were calculated using admetSAR2.0 (Yang et al 2019).

**Results**

**Prediction of targets**

Andrographolide was predicted to regulate down-regulated 36 proteins in which 17 were down-regulated and 19 were upregulated. Likewise, 14-Deoxy-11,12-didehydroandrographolide regulated 48 proteins in which 21 were downregulated and 27 were upregulated. The list of regulated proteins with their probable activity and inactivity of both compounds is summarized in Table 1.

**Enrichment analysis**

Seventy-two different pathways were identified to be regulated by the Andrographolide in which pathways in cancer were primarily regulated by modulating nine genes i.e. AR, ESR2, IL6R, MDM2, PRKCA, RAC1, RARA, RHOA, RXRA at the false discovery rate of 4.96E-05. Similarly, 14-Deoxy-11,12-
didehydroandrographolide was predicted to regulate seventy-seven different pathways by modulating the Estrogen signaling pathway via seven genes i.e. ESR2, FKBP5, KRT16, KRT17, KRT18, PGR, RARA at the false discovery rate of 7.57E-06. Pathways modulated by Andrographolide and 14-Deoxy-1,12-didehydroandrographolide with their respective genes are summarized in Table 2 and Table 3 respectively. Similarly, the interaction of both compounds with the proteins from regulated pathways is represented in Figures 1 and 2. Further, the number of genes in multiple cellular components, biological process, and molecular function is for Andrographolide and 14-Deoxy-1,12-didehydroandrographolide are represented in Figure 3 and Figure 4, respectively. Similarly, network analysis of 14-Deoxy-1,12-didehydroandrographolide identified prime regulation of PRKCA protein and estrogen signaling pathway. Further, andrographolide primarily modulated PRKCA protein and pathways in cancer.

**In silico molecular docking**

Among Andrographolide and 14-Deoxy-1,12-didehydroandrographolide, 14-Deoxy-1,12-didehydroandrographolide was predicted to have the highest binding affinity with papain-like protease i.e. -6.7 kcal/mol; however, it was not having any hydrogen bond interactions. Similarly, andrographolide showed -6.5 kcal/mol binding energy with papain-like protein with one hydrogen bond interaction i.e. TYR274. Although both molecules were having equal binding energy with 3clpro (-6.8 kcal/mol), the number of hydrogen bond interactions were more in andrographolide due to interaction with THR190, HIS163, and CYS145. Further, both molecules showed a binding affinity with spike protein i.e. -6.9 kcal/mol; however, andrographolide showed one hydrogen bond interaction with LYS807 (Table 4). The interaction of each compound with respective proteins is represented in Figure 5.

**Drug-likeness and ADMET profiling**

14-Deoxy-1,12-didehydroandrographolide scored higher druglikeness score i.e. -0.52 compared to andrographolide which was computed based on molecular weight, number of hydrogen bond donor, number of hydrogen bond acceptor and LogP value (Table 5) which has influenced the pharmacokinetic characters of both compounds by affecting absorption, distribution, metabolism, excretion, and toxicity (Figure 6).

**Discussion**

The present study dealt to investigate one of the active biomolecules andrographolide and its derivative 14-Deoxy-1,12-didehydroandrographolide from *Andrographis paniculata* to modulate the proteins/pathways for immunomodulatory activity and their binding affinity with three proteins i.e. 3clpro, PLpro and spike protein which are the targets of COVID infection. Further, we investigated the drug-likeness character of both molecules in which andrographolide scored lower drug-likeness character compared to its derivative. However, on looking to the binding affinity and number of hydrogen bond interactions, andrographolide showed a higher affinity towards the selected targets. This suggests, fewer modifications could be made in the andrographolide moiety to enhance the drug-likeness character without altering the binding affinity of the molecules.
Subjects with lower immunity systems are identified to be more prone towards the infection with COVID-19 due to compromised immunity systems (Science daily, 2020) which can be well visualized in the subjects who are suffering from an infectious and non-infectious disease. In this case, it is important to enhance the immunity of the subjects to minimize the probability of viral infection. In the present study via the enrichment analysis, we identified pathways that are involved to boost the immune system which is modulated by andrographolide and its derivative.

In the present study, we identified modulation of few pathways that are directly or indirectly linked with the modulation of the immune system i.e. Chemokine signaling pathway, Rap1 signaling pathway, Cytokine-cytokine receptor interaction, MAPK signaling pathway, NF-kappa B signaling pathway, Rassignaling pathway, p53 signaling pathway, HIF-1 signaling pathway, and Natural killer cell-mediated cytotoxicity. Among the above pathways, Chemokine signaling pathways, Rap1 signaling pathway, and Cytokine-cytokine receptor interaction are the choice of interest pathways as they are directly linked with the modulation of the immune system and they scored minimum false discovery rate compared to rest of the pathways. Chemokine signaling pathway was found to be modulated by andrographolide and its derivative which could contribute to controlling the migration of immune cells in tissues (Sokol and Luster, 2015). Further, Rap1 signaling pathway is involved in activating three secondary messengers i.e. cAMP, calcium, and diacylglycerol (Kortlever et al., 2017) which are needed in the signaling of cell position during viral infections; modulated by andrographolide by regulating ID1, PRKCA, RAC1, RAP1A, and RHOA and by 14-deoxy-11,12-didehydroandrographolide by regulating FLT1, ID1, PRKCA, RAC1, RAP1A, and RHOA. Similarly, cytokine-cytokine receptor interaction has been recorded by the KEGG database as an entry (hsa04060) in various auto-immune disorders. Since COVID-19 has a more risk over the infections on altered immune system of subjects, modulation of this pathway could be beneficial in them which has been modulated by andrographolide and its derivative. Further, the MAPK signaling pathway has been identified to play important role in the functioning of T lymphocytes (Chi and Flavell, 2010), was observed to be modulated by andrographolide and its derivative. Additionally, other pathways like NF-kappa B signaling pathway, Rassignaling pathway, p53 signaling pathway, HIF-1 signaling pathway, and Natural killer cell-mediated cytotoxicity are also regulated which has been well reported to be involved in the modulation of the immune system.

In COVID-19 infection, the n-CoV-2 binds to ACE-2 and enters into the cell and starts deregulating the intracellular functions by altering the normal homeostatic stimulus (Magrone et al., 2020). Hence, it is needed to have control over the components by binding over them or responding towards the stimulus COVID-19 main protease, or at least to minimize its effect by controlling the intracellular cascade initiated by the viral infection. In the present study, GO enrichment analysis identified andrographolide and 14-Deoxy-11,12-didehydroandrographolide were also predicted to majorly target the intracellular components, binding capacity towards various proteins as a molecular function and responder towards stimulus which could be the probable action of these two agents over the viral infection.

A concept of modulation of multiple proteins by a single molecule is the choice of research interest in identifying the lead hit and their respective targets. Further, andrographolide has been previously reported
to possess anti-viral property (Gupta et al 2017). Hence, based on the same concept, andrographolide and its derivative may also possess the anti-viral efficacy over COVID-19 which kindled us evaluating the binding affinity of these bioactives over andrographolidePLpro, 3clpro, and spike protein. Although the drug-likeness score model predicted 14-Deoxy-11,12-didehydroandrographolide to behave like a drug based on “Rule of Five”, its binding affinity and number of hydrogen bond interactions showed andrographolide to inhibit more on three proteins of COVID-19 i.e. Papain-like protease (PLpro), 3clpro, and spike protein.

**Conclusion**

The present study dealt to utilize the system biology approach to investigate the andrographolide and its derivative against COVID-19 by modulating the multiple pathways in which the Chemokine signaling pathway could be a choice of interest as it is directly linked to the modulation of the immune system with lowest false discovery rate. Further, andrographolide could possess higher importance compared to its derivate 14-Deoxy-11,12-didehydroandrographolide as it scored higher interaction with the targeted proteins of COVID-19.

**Declarations**

**Competing interest:** The authors declare no competing interest.

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Tables

Table 1: Regulated proteins by andrographolide and 14-Deoxy-11,12-didehydroandrographolide
| Pa   | Pi  | Modulated proteins | Pa   | Pi  | Modulated proteins | Pa   | Pi  | Modulated proteins |
|------|-----|--------------------|------|-----|--------------------|------|-----|--------------------|
| 0.548| 0.132| TOP2A              | 0.589| 0.131| VDR               | 0.66 | 0.117| CHEK1             |
| 0.559| 0.163| CHEK1              | 0.526| 0.082| CD14              | 0.627| 0.097| TOP2A             |
| 0.387| 0.028| KRT16             | 0.336| 0.079| CLU               | 0.562| 0.041| IFL               |
| 0.364| 0.038| KRT17             | 0.444| 0.198| AR                | 0.451| 0.016| KRT16             |
| 0.331| 0.026| PTH               | 0.417| 0.175| ID1               | 0.445| 0.025| KRT17             |
| 0.394| 0.138| ESR2             | 0.231| 0.043| RAP1A            | 0.379| 0.015| PTH               |
| 0.297| 0.076| TIMP2             | 0.375| 0.197| RAC1             | 0.452| 0.092| ESR2             |
| 0.38 | 0.161| CCL2             | 0.32 | 0.17 | GPX1             | 0.474| 0.127| MDM2             |
| 0.38 | 0.161| IVL              | 0.241| 0.092| KLK2            | 0.444| 0.097| CCL2             |
| 0.306| 0.142| LEP               | 0.394| 0.257| NPPB             | 0.34 | 0.044| TIMP2        |
| 0.364| 0.212| PRKCA            | 0.319| 0.186| TNFRSF1A        | 0.357| 0.086| LEP         |
| 0.3  | 0.155| CCL4             | 0.31 | 0.191| KRT18         | 0.403| 0.139| PRKCA         |
| 0.27 | 0.141| IL6R             | 0.171| 0.058| RXRA         | 0.328| 0.107| CCL4         |
| 0.226| 0.138| GYPA             | 0.356| 0.248| RARA         | 0.382| 0.187| NR3C1         |
| 0.349| 0.269| MDM2             | 0.205| 0.103| RHOB         | 0.299| 0.105| IL6R         |
| 0.319| 0.28  | NR3C1           | 0.178| 0.095| RHOA         | 0.384| 0.206| CASP8         |
| 0.221| 0.206| CD44             | 0.354| 0.328| CD83         | 0.139| 0.051| PTHHL         |
| 0.294| 0.291| SMN2             | 0.247| 0.166| CD44         | 0.362| 0.293| NOS2         |
| 0.191| 0.19  | CD38            | 0.276| 0.229| FLT1         | 0.224| 0.065| RHOB         |
| 0.255| 0.231| PROS1            | 0.195| 0.04  | RXRA         | 0.2 | 0.057  | RHOB         |
|      |      |                  |      |      |                  |      |      |                  |

Table 2: Enrichment analysis of andrographolide

Pa: probable activity, Pi: Probable inactivity
| #term ID | term description                                           | observed gene count | false discovery rate | matching proteins in network (labels) |
|---------|-------------------------------------------------------------|---------------------|----------------------|--------------------------------------|
| hsa05200 | Pathways in cancer                                          | 9                   | 4.96E-05             | AR, ESR2, IL6R, MDM2, PRKCA, RAC1, RARA, RHOA, RXRA |
| hsa04640 | Hematopoietic cell lineage                                  | 5                   | 7.61E-05             | CD14, CD38, CD44, GYPA, IL6R        |
| hsa04972 | Pancreatic secretion                                        | 5                   | 7.61E-05             | CD38, PRKCA, RAC1, RAP1A, RHOA     |
| hsa05130 | Pathogenic Escherichia coli infection                       | 4                   | 0.00014              | CD14, KRT18, PRKCA, RHOA           |
| hsa04915 | Estrogen signaling pathway                                  | 5                   | 0.00016              | ESR2, KRT16, KRT17, KRT18, RARA    |
| hsa04932 | Non-alcoholic fatty liver disease (NAFLD)                   | 5                   | 0.00022              | IL6R, LEP, RAC1, RXRA, TNFRSF1A    |
| hsa04062 | Chemokine signaling pathway                                 | 5                   | 0.00048              | CCL2, CCL4, RAC1, RAP1A, RHOA     |
| hsa05205 | Proteoglycans in cancer                                     | 5                   | 0.00059              | CD44, MDM2, PRKCA, RAC1, RHOA     |
| hsa04015 | Rap1 signaling pathway                                      | 5                   | 0.00063              | ID1, PRKCA, RAC1, RAP1A, RHOA     |
| hsa04670 | Leukocyte transendothelial migration                        | 4                   | 0.00091              | PRKCA, RAC1, RAP1A, RHOA           |
| hsa04071 | Sphingolipid signaling pathway                              | 4                   | 0.00095              | PRKCA, RAC1, RHOA, TNFRSF1A       |
| hsa04060 | Cytokine-cytokine receptor interaction                      | 5                   | 0.0013               | CCL2, CCL4, IL6R, LEP, TNFRSF1A    |
| hsa04961 | Endocrine and other factor-regulated calcium reabsorption   | 3                   | 0.0013               | KLK2, PRKCA, VDR                   |
| hsa05014 | Amyotrophic lateral sclerosis (ALS)                         | 3                   | 0.0013               | GPX1, RAC1, TNFRSF1A               |
| hsa05418 | Fluid shear stress and atherosclerosis                      | 4                   | 0.0013               | CCL2, RAC1, RHOA, TNFRSF1A         |
| hsa05206 | MicroRNAs in cancer                                         | 4                   | 0.0017               | CD44, MDM2, PRKCA, RHOA           |
| hsa04010 | MAPK signaling pathway                                      | 5                   | 0.0018               | CD14, PRKCA, RAC1, RAP1A, TNFRSF1A |
| hsa04920 | Adipocytokine signaling pathway                             | 3                   | 0.0024               | LEP, RXRA, TNFRSF1A               |
| hsa05152 | Tuberculosis                                                | 4                   | 0.0024               | CD14, RHOA, TNFRSF1A,VDR           |
| hsa05202 | Transcriptional misregulation in cancer                     | 4                   | 0.0024               | CD14, MDM2, RARA, RXRA            |
| hsa05203 | Viral carcinogenesis                                        | 4                   | 0.0027               | CHEK1, MDM2, RAC1, RHOA           |
| hsa04151 | PI3K-Akt signaling pathway                                  | 5                   | 0.0031               | IL6R, MDM2, PRKCA, RAC1, RXRA    |
| hsa04510 | Focal adhesion                                              | 4                   | 0.0033               | PRKCA, RAC1, RAP1A, RHOA           |
| hsa05132 | Salmonella infection                                        | 3                   | 0.0035               | CCL4, CD14, RAC1                  |
| hsa04064 | NF-kappa B signaling pathway                                | 3                   | 0.0045               | CCL4, CD14, TNFRSF1A              |
| hsa04014 | Ras signaling pathway                                       | 4                   | 0.005                | PRKCA, RAC1, RAP1A, RHOA          |
| hsa04933 | AGE-RAGE signaling pathway in diabetic complications         | 3                   | 0.005                | CCL2, PRKCA, RAC1                  |
| hsa04620 | Toll-like receptor signaling pathway                        | 3                   | 0.0052               | CCL4, CD14, RAC1                  |
| hsa04659 | Th17 cell differentiation                                   | 3                   | 0.0052               | IL6R, RARA, RXRA                  |
| hsa04722 | Neurotrophin signaling pathway                              | 3                   | 0.0067               | RAC1, RAP1A, RHOA                 |
| hsa04919 | Thyroid hormone signaling pathway                           | 3                   | 0.0067               | MDM2, PRKCA, RXRA                 |
| hsa04310 | Wnt signaling pathway                                       | 3                   | 0.0116               | PRKCA, RAC1, RHOA                |
| hsa04150 | mTOR signaling pathway                                      | 3                   | 0.0124               | PRKCA, RHOA, TNFRSF1A             |
| hsa04921 | Oxytocin signaling pathway                                  | 3                   | 0.0124               | CD38, PRKCA, RHOA                 |
| hsa04530 | Tight junction                                              | 3                   | 0.0161               | RAC1, RAP1A, RHOA                 |
Table 3: Enrichment analysis of 14-deoxy-11,12-didehydroandrographolide

| Enriched Pathway                                      | FDR   | DEGs                                    |
|-------------------------------------------------------|-------|-----------------------------------------|
| Malaria                                               | 0.0161| CCL2,GYPA                               |
| Influenza A                                           | 0.0161| CCL2,PRKCA,TNFRSF1A                     |
| Axon guidance                                         | 0.0166| PRKCA,RAC1,RHOA                         |
| cAMP signaling pathway                                | 0.0221| RAC1,RAP1A,RHOA                         |
| VEGF signaling pathway                                | 0.0221| PRKCA,RAC1                              |
| Epstein-Barr virus infection                          | 0.0221| CD38,CD44,MDM2                         |
| Long-term potentiation                                | 0.0233| PRKCA,RAP1A                             |
| Regulation of actin cytoskeleton                      | 0.0233| CD14,RAC1,RHOA                         |
| Shigelllosis                                          | 0.0233| CD44,RAC1                               |
| Platinum drug resistance                              | 0.0237| MDM2,TOP2A                             |
| p53 signaling pathway                                 | 0.0237| CHEK1,MDM2                             |
| Adherens junction                                     | 0.0237| RAC1,RHOA                              |
| Fc epsilon RI signaling pathway                       | 0.0237| PRKCA,RAC1                              |
| Bacterial invasion of epithelial cells                | 0.0237| RAC1,RHOA                              |
| Renal cell carcinoma                                  | 0.0237| RAC1,RAP1A                             |
| Glioma                                                | 0.0237| MDM2,PRKCA                             |
| Acute myeloid leukemia                                | 0.0237| CD14,RARA                              |
| Non-small cell lung cancer                            | 0.0237| PRKCA,RXRA                             |
| Thyroid hormone synthesis                             | 0.0239| GPX1,PRKCA                             |
| Pertussis                                             | 0.0240| CD14,RHOA                              |
| EGFR tyrosine kinase inhibitor resistance             | 0.0260| IL6R,PRKCA                             |
| TGF-beta signaling pathway                            | 0.0287| ID1,RHOA                               |
| Colorectal cancer                                     | 0.0295| RAC1,RHOA                              |
| Salivary secretion                                    | 0.0297| CD38,PRKCA                             |
| Fc gamma R-mediated phagocytosis                      | 0.0311| PRKCA,RAC1                              |
| Amoebiasis                                            | 0.0339| CD14,PRKCA                             |
| Endocrine resistance                                  | 0.0340| ESR2,MDM2                              |
| Prostate cancer                                       | 0.0348| AR,MDM2                                |
| HIF-1 signaling pathway                               | 0.0349| IL6R,PRKCA                             |
| Choline metabolism in cancer                          | 0.0349| PRKCA,RAC1                              |
| Chagas disease (American trypanosomiasis)             | 0.0358| CCL2,TNFRSF1A                          |
| TNF signaling pathway                                 | 0.0399| CCL2,TNFRSF1A                          |
| Vascular smooth muscle contraction                    | 0.0470| PRKCA,RHOA                             |
| Cell cycle                                            | 0.0492| CHEK1,MDM2                             |
| Osteoclast differentiation                            | 0.0492| RAC1,TNFRSF1A                          |
| Platelet activation                                   | 0.0492| RAP1A,RHOA                             |
| Natural killer cell mediated cytotoxicity             | 0.0492| PRKCA,RAC1                             |
| #term ID | term description                              | observed gene count | false discovery rate | matching proteins in network                              |
|----------|----------------------------------------------|---------------------|----------------------|----------------------------------------------------------|
| hsa04915 | Estrogen signaling pathway                   | 7                   | 7.57E-06             | ESR2, FKBP5, KRT16, KRT17, KRT18, PCG, RARA              |
| hsa05200 | Pathways in cancer                           | 11                  | 7.57E-06             | AR, CASP8, ESR2, IL6R, MDM2, NOS2, PRKCA, RAC1, RARA, RHOA, RXRA |
| hsa05202 | Transcriptional misregulation in cancer      | 7                   | 1.32E-05             | CD14, FLTI, MDM2, PLAT, PLAU, RARA, RXRA                |
| hsa04932 | Non-alcoholic fatty liver disease (NAFLD)    | 6                   | 9.13E-05             | CASP8, IL6R, LEP, RAC1, RXRA, TNFRSF1A                  |
| hsa05152 | Tuberculosis                                 | 6                   | 0.00016              | CASP8, CD14, NOS2, RHOA, TNFRSF1A, VDR                 |
| hsa04015 | Rap1 signaling pathway                       | 6                   | 0.00023              | FLT1, ID1, PRKCA, RAC1, RAP1A, RHOA                    |
| hsa05014 | Amyotrophic lateral sclerosis (ALS)          | 4                   | 0.00023              | CAT, GPK1, RAC1, TNFRSF1A                               |
| hsa05130 | Pathogenic Escherichia coli infection        | 4                   | 0.00023              | CD14, KRT18, PRKCA, RHOA                               |
| hsa05205 | Proteoglycans in cancer                     | 6                   | 0.00023              | CD44, MDM2, PLAU, PRKCA, RAC1, RHOA                    |
| hsa05418 | Fluid shear stress and atherosclerosis      | 6                   | 0.00035              | CCL2, PLAT, RAC1, RHOA, TNFRSF1A                       |
| hsa05206 | MicroRNAs in cancer                          | 5                   | 0.00054              | CD44, MDM2, PLAU, PRKCA, RHOA                          |
| hsa04060 | Cytokine-cytokine receptor interaction      | 6                   | 0.00065              | CCL2, CCL4, FLTI, IL6R, LEP, TNFRSF1A                  |
| hsa04610 | Complement and coagulation cascades          | 4                   | 0.00065              | CLU, PLAT, PLAU, PROS1                                 |
| hsa05132 | Salmonella infection                         | 4                   | 0.00075              | CCL4, CD14, NOS2, RAC1                                 |
| hsa04010 | MAPK signaling pathway                       | 6                   | 0.00095              | CD14, FLTI, PRKCA, RAC1, RAP1A, TNFRSF1A               |
| hsa04062 | Chemokine signaling pathway                 | 5                   | 0.00095              | CCL2, CCL4, RAC1, RAP1A, RHOA                           |
| hsa04064 | NF-kappa B signaling pathway                 | 4                   | 0.00095              | CCL4, CD14, PLAU, TNFRSF1A                              |
| hsa04066 | HIF-1 signaling pathway                      | 4                   | 0.00095              | FLT1, IL6R, NOS2, PRKCA                                |
| hsa04640 | Hematopoietic cell lineage                   | 4                   | 0.00095              | CD14, CD38, CD44, IL6R                                 |
| hsa05203 | Viral carcinogenesis                         | 5                   | 0.00095              | CASP8, CHEK1, MDM2, RAC1, RHOA                         |
| hsa05215 | Prostate cancer                              | 4                   | 0.00095              | AR, MDM2, PLAT, PLAU                                   |
| hsa04510 | Focal adhesion                               | 5                   | 0.00097              | FLT1, PRKCA, RAC1, RAP1A, RHOA                         |
| hsa04620 | Toll-like receptor signaling pathway         | 4                   | 0.00097              | CASP8, CCL4, CD14, RAC1                                |
| hsa05142 | Chagas disease (American trypanosomiasis)   | 4                   | 0.00097              | CASP8, CCL2, NOS2, TNFRSF1A                             |
| hsa04670 | Leukocyte transendothelial migration        | 4                   | 0.0013               | PRKCA, RAC1, RHOA, TXRA                                |
| hsa04071 | Sphingolipid signaling pathway              | 4                   | 0.0014               | PRKCA, RAC1, RHOA, TNFRSF1A                             |
| hsa04151 | PI3K-Akt signaling pathway                   | 6                   | 0.0014               | FLT1, IL6R, MDM2, PRKCA, RAC1, RXRA                    |
| hsa04014 | Ras signaling pathway                        | 5                   | 0.0015               | FLT1, PRKCA, RAC1, RAP1A, RHOA                         |
| hsa04961 | Endocrine and other factor-regulated calcium reabsorption | 3                   | 0.0015               | KLK2, PRKCA, VDR                                      |
| hsa04115 | p53 signaling pathway                        | 3                   | 0.004                | CASP8, CHEK1, MDM2                                    |
| hsa04920 | Adipocytokine signaling pathway              | 3                   | 0.004                | LEP, RXRA, TNFRSF1A                                    |
| hsa01524 | Platinum drug resistance                     | 3                   | 0.0041               | CASP8, MDM2, TOP2A                                     |
| ID    | Pathway                                                      | Count | p-value  | Genes                                                                 |
|-------|--------------------------------------------------------------|-------|----------|-----------------------------------------------------------------------|
| hsa04621 | NOD-like receptor signaling pathway                           | 4     | 0.0041   | CASP8, CCL2, CTSB, RHOA                                              |
| hsa05133 | Pertussis                                                    | 3     | 0.0045   | CD14, NO52, RHOA                                                      |
| hsa05146 | Amoebiasis                                                   | 3     | 0.0085   | CD14, NO52, PRKCA                                                     |
| hsa04933 | AGE-RAGE signaling pathway in diabetic complications          | 3     | 0.0093   | CCL2, PRKCA, RAC1                                                    |
| hsa04659 | Th17 cell differentiation                                    | 3     | 0.0101   | IL6R, RARA, RXRA                                                     |
| hsa04668 | TNF signaling pathway                                        | 3     | 0.0115   | CASP8, CCL2, TNFRSF1A                                                |
| hsa05145 | Toxoplasmosis                                                | 3     | 0.0115   | CASP8, NO52, TNFRSF1A                                                |
| hsa04933 | AGE-RAGE signaling pathway in diabetic complications          | 3     | 0.0093   | CCL2, PRKCA, RAC1                                                    |
| hsa04215 | Apoptosis - multiple species                                 | 2     | 0.0127   | CASP8, TNFRSF1A                                                      |
| hsa04722 | Neurotrophin signaling pathway                               | 3     | 0.0127   | RAC1, RAP1A, RHOA                                                    |
| hsa04919 | Thyroid hormone signaling pathway                            | 3     | 0.0127   | MDM2, PRKCA, RXRA                                                    |
| hsa04210 | Apoptosis                                                    | 3     | 0.0188   | CASP8, CTSB, TNFRSF1A                                                |
| hsa04310 | Wnt signaling pathway                                        | 3     | 0.0215   | PRKCA, RAC1, RHOA                                                    |
| hsa04150 | mTOR signaling pathway                                       | 3     | 0.0231   | PRKCA, RHOA, TNFRSF1A                                                |
| hsa04921 | Oxytocin signaling pathway                                   | 3     | 0.0306   | CD38, PRKCA, RHOA                                                    |
| hsa04370 | VEGF signaling pathway                                       | 2     | 0.0308   | CASP8, RAC1                                                          |
| hsa04360 | Axon guidance                                                | 3     | 0.0311   | PRKCA, RAC1, RHOA                                                    |
| hsa04370 | VEGF signaling pathway                                       | 2     | 0.0327   | PRKCA, RAC1                                                          |
| hsa04020 | Calcium signaling pathway                                    | 3     | 0.0328   | CD38, NO52, PRKCA                                                    |
| hsa05168 | Herpes simplex infection                                     | 3     | 0.0332   | CASP8, CCL2, TNFRSF1A                                                |
| hsa05167 | Kaposi's sarcoma-associated herpesvirus infection            | 3     | 0.0335   | CASP8, RAC1, TNFRSF1A                                                |
| hsa05311 | Viral myocarditis                                           | 2     | 0.0343   | CD44, RAC1                                                           |
| hsa04720 | Long-term potentiation                                       | 2     | 0.0347   | PRKCA, RAP1A                                                         |
| hsa0424  | cAMP signaling pathway                                       | 3     | 0.0362   | RAC1, RAP1A, RHOA                                                    |
| hsa04664 | Fc epsilon RI signaling pathway                              | 2     | 0.0362   | PRKCA, RAC1                                                          |
| hsa05169 | Epstein-Barr virus infection                                 | 3     | 0.0362   | CD38, CD44, MDM2                                                     |
| hsa05211 | Renal cell carcinoma                                         | 2     | 0.0362   | RAC1, RAP1A                                                          |
| hsa05214 | Glioma                                                       | 2     | 0.0362   | MDM2, PRKCA                                                          |
| hsa05221 | Acute myeloid leukemia                                       | 2     | 0.0362   | CD14, RARA                                                           |
| hsa05223 | Non-small cell lung cancer                                   | 2     | 0.0362   | PRKCA, RXRA                                                          |
| hsa04520 | Adherens junction                                            | 2     | 0.037    | RAC1, RHOA                                                           |
| hsa04810 | Regulation of actin cytoskeleton                             | 3     | 0.037    | CD14, RAC1, RHOA                                                    |
| hsa04918 | Thyroid hormone synthesis                                   | 2     | 0.037    | GPX1, PRKCA                                                          |
| hsa04976 | Bile secretion                                              | 2     | 0.037    | CYP3A4, RXRA                                                         |
| hsa05100 | Bacterial invasion of epithelial cells                       | 2     | 0.037    | RAC1, RHOA                                                           |
| hsa01521 | EGFR tyrosine kinase inhibitor resistance                    | 2     | 0.0409   | IL6R, PRKCA                                                          |
| hsa04146 | Peroxisome                                                   | 2     | 0.0433   | CAT, NO52                                                            |
| hsa04350 | TGF-beta signaling pathway                                   | 2     | 0.0446   | ID1, RHOA                                                            |
| hsa05323 | Rheumatoid arthritis                                         | 2     | 0.045    | CCL2, FLT1                                                           |
| hsa05210 | Colorectal cancer                                            | 2     | 0.0454   | RAC1, RHOA                                                           |
| hsa04970 | Salivary secretion                                           | 2     | 0.0457   | CD38, PRKCA                                                          |
| hsa04666 | Fc gamma R-mediated phagocytosis                             | 2     | 0.0481   | PRKCA, RAC1                                                          |
Table 4: Docking hits of andrographolide and 14-deoxy-11,12-didehydroandrographolide with Plpro, 3clpro and spike protein

| Targets                  | Ligand                          | Binding Affinity (kcal/mol) | Number of hydrogen bonds | Hydrogen bond residues   |
|-------------------------|---------------------------------|----------------------------|--------------------------|--------------------------|
| Papain-like protease (PLpro) | 14-deoxy-11,12-didehydroandrographolide | -6.7                      | -                        | -                        |
| 4M0W                    | andrographolide                 | -6.5                      | 1                        | TYR274                   |
| 3clpro                  | 14-deoxy-11,12-didehydroandrographolide | -6.8                      | 1                        | ARG131                   |
| 6LU7                    | andrographolide                 | -6.8                      | 3                        | THR190, HIS163, CYS145   |
| Spike protein           | 14-deoxy-11,12-didehydroandrographolide | -6.9                      | -                        | -                        |
|                         | andrographolide                 | -6.9                      | 1                        | LYS807                   |

Table 5: Druglikeness score of andrographolide and 14-deoxy-11,12-didehydroandrographolide

|                        | andrographolide | 14-deoxy-11,12-didehydroandrographolide |
|------------------------|-----------------|------------------------------------------|
| Molecular formula      | C_{20}H_{30}O_{5} | C_{20}H_{32}O_{4}                        |
| Molecular weight       | 350.21          | 332.20                                   |
| Number of HBA          | 5               | 4                                        |
| Number of HBD          | 3               | 2                                        |
| MolLogP                | 2.19            | 3.09                                     |
| MolLogS                | -1.97           | -2.67                                    |
| Log(moles/L) mg/L      | 3791.12         | 702.11                                   |
| MolPSA (Å^2)           | 71.27           | 55.16                                    |
| MolVol (Å^3)           | 416.03          | 421.79                                   |
| Number of stereo centers | 6             | 5                                        |
| Drug-likeness model score | -0.64       | -0.52                                    |

Figures
Figure 1

Network interaction of andrographolide with its targets and probably modulated pathways
Figure 2

Network interaction of 14-Deoxy-11,12-didehydroandrographolide with its targets and probably modulated pathways
Figure 3

GO analysis for andrographolide (a) cellular component, (b) molecular function, and (c) biological process
Figure 4

GO analysis for andrographolide (a) cellular component, (b) molecular function, and (c) biological process
Figure 5

Interaction of (a) Andrographolide and (b) 14-Deoxy-11,12-didehydroandrographolide with (1) PLpro, (2) 3clpro and (3) spike protein
Figure 6

ADMET (a) Andrographolide, (b) 14-Deoxy-11,12-didehydroandrographolide Red:lower, Green: High