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A NETWORK PHARMACOLOGY-BASED INVESTIGATION ON THE POTENTIAL EFFECT OF MORINGA OLEIFERA AND GEMCITABINE COMBINATION AGAINST PANCREATIC CANCER

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ABSTRACT

A NETWORK PHARMACOLOGY-BASED INVESTIGATION ON THE POTENTIAL EFFECT OF MORINGA OLEIFERA AND GEMCITABINE COMBINATION AGAINST PANCREATIC CANCER

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Introduction: Pancreatic cancer is lethal and often considered as metastatic disease by the time of diagnosis as the signs and symptoms are difficult to detect. Gemcitabine (GEM) is the first-line chemotherapy drug against patients with advanced pancreatic cancer. *Moringa oleifera* (MO), a well-known Ayurvedic Indian medicine which is commonly found in India, Africa and Southeast Asia has exhibited various biological activity including anti-cancer effects, particularly on the pancreatic cancer cells. However, the effectiveness of MO and GEM combination against pancreatic cancer has yet to be explored. **Objective:** This study aims to evaluate the potential effect of MO together with GEM against pancreatic cancer by the integration of network pharmacology. **Methodology:** Traditional Chinese Medicine System Pharmacology, Traditional Chinese Medicine Integrated Database and PubMed databases were used to identify and screen the bioactive compounds in MO. Target genes of MO and GEM were predicted through Drug Gene Interaction Database, Comparative Toxicogenomics Database, and DrugBank databases. Pancreatic cancer genes were collected from Online Mendelian Inheritance in Man and MalaCards databases. Venn diagrams were constructed to identify pancreatic cancer-related target genes using Bioinformatics and Evolutionary Genomics tools. Protein-protein interaction (PPI) and compound-target-pathway network were established via STRING and Cytoscape, respectively. Gene ontology (GO) and pathway enrichment analysis were conducted using DAVID Bioinformatic Tools. **Results:** A total of 32 compounds have been

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identified in MO. Catechin, kaempferol, quercetin and epicatechin that met the drug screening requirements and 3 additional compounds- glucomoringin, glucoraphanin and moringinine were identified as bioactive compounds. Catechin was found to be the main hub compound in MO. There are 1092, 352 and 421 target genes were found in MO, GEM and pancreatic cancer, respectively. The Venn diagram revealed 5 intersections between the combination of MO and GEM. Among those intersections, 4 intersections were investigated which are GEM-, MO-, MO+GEM- and shared biotargets-intersections against pancreatic cancer. The studied compounds stimulate 4 hub genes from PPI network, which include *TP53, AKT1, VEGFA* and *CCND1* in targeting pancreatic cancer. Also, 2 hub genes were identified including *CASP3 and BCL2L1* which may represent the new targets against pancreatic cancer as they are not targeted by MO or GEM alone. GO and pathway analysis revealed that MO and GEM combination was mainly associated with cancer including pancreatic cancer through regulation of apoptosis and cell proliferation. **Discussion:** This is the first network pharmacology study that predict the target genes of the bioactive compounds of MO in combination with GEM and theoretically evaluate their effects against pancreatic cancer. Hub genes play a crucial role within the network with high biological importance and significantly enriched in multiple pathways. Thus, this study has revealed the multi-compounds, multi-targets and multi-pathways of MO and GEM combination against pancreatic cancer. **Conclusion:** To conclude, this *in silico* study suggested that the combination therapy of MO bioactive compounds and GEM synergistically enhance the effect in pancreatic cancer treatment. However, further experimental research and subsequent clinical applications are needed to validate these findings.

Keywords: Moringa oleifera; pancreatic cancer; bioactive compound; gemcitabine; network pharmacology

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ABSTRAK

PENYIASATAN BERDASARKAN FARMAKOLOGI RANGKAIAN TERHADAP KESAN POTENSI GABUNGAN MORINGA OLEIFERA DAN GEMCITABINE KE ATAS BARAH PANKREAS

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Pengenalan: Barah pankreas ialah penyakit yang membawa maut dan sering dianggap sebagai metastatik ketika diagnosis kerana tanda dan gejala amat sukar untuk dikesan. Gemcitabine (GEM) merupakan ubat kemoterapi barisan pertama terhadap pesakit barah pankreas. *Moringa oleifera* (MO), ubat Ayuverda India terkenal yang biasa dijumpai di India, Afrika dan Asia Tenggara telah menunjukkan pelbagai aktiviti biologi termasuk kesan anti barah terutama pada sel barah pankreas. Namun, keberkesanan penggabungan MO dan GEM terhadap barah pankreas masih belum diterokai. **Objektif:** Kajian ini bertujuan untuk menilai kesan penggabungan MO bersama-sama dengan GEM terhadap barah pankreas dengan penyatuan farmakologi rangkaian. **Metodologi:** Pangkalan data "Traditional Chinese Medicine System Pharmacology", "Traditional Chinese Medicine Integrated Database" dan "PubMed" digunakan bagi mengenal pasti dan menyaring sebatian bioaktif dalam MO. Gen sasaran MO dan GEM diramalkan melalui pangkalan data "Drug Gene Interaction Database", "Comparative Toxicogenomics Database" dan "DrugBank". Gen barah pankreas diambil dari pangkalan data "Online Mendelian Inheritance in Man" dan "MalaCards". Gambar rajah Venn dihasilkan bagi mengenal pasti gen sasaran barah pankreas dengan menggunakan alat "Bioinformatics and Evolutionary Genomics". Interaksi protein-protein (PPI) dan rangkaian sebatian-sasaran-laluan masing-masing dihasilkan melalui "STRING" dan "Cytoscape". Analisis ontologi gen (GO) dan pengkayaan laluan dilakukan dengan menggunakan alat bioinformatik DAVID. **Keputusan:** Sebanyak 32 sebatian telah dikenal pasti dalam MO. Catechin,

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kaempferol, quercetin dan epicatechin yang memenuhi syarat pemeriksaan ubat dan 3 sebatian tambahan- glukomoringin, glucoraphanin dan moringinin dikenal pasti sebagai sebatian bioaktif. Catechin merupakan sebatian hub utama dalam MO. Terdapat 1092, 352 dan 421 gen sasaran masing-masing dijumpai pada MO, GEM dan barah pankreas. Gambar rajah Venn menunjukkan 5 persilangan antara gabungan MO dan GEM. Di antara persilangan tersebut, 4 persilangan telah disiasat iaitu GEM-, MO-, MO+GEM- dan persilangan biotarget yang dikongsi terhadap barah pankreas. Sebatian yang dikaji merangsang 4 gen hub dari rangkaian PPI, yang merangkumi *TP53, AKT1, VEGFA* dala *CCND1* dalam mensasarkan barah pankreas. Dua gen juga telah dikenal pasti termasuk *CASP3* dan *BCL2L1* yang mungkin mewakili sasaran baru terhadap barah pankreas kerana ianya tidak disasarkan oleh MO atau GEM sahaja. Analisis GO dan laluan menunjukkan bahawa penggabungan MO dan GEM berkait dengan barah terutamanya, termasuk barah pankreas, melalui pengaturan apoptosis. **Perbincangan:** Kajian ini merupakan farmakologi rangkaian pertama yang meramalkan gen sasaran gabungan sebatian bioaktif MO dan GEM serta menilai kesan terhadap barah pankreas secara teori. Gen hub memainkan peranan penting dalam rangkaian dengan kepentingan biologi yang tinggi dan diperkaya secara signifikan dalam pelbagai laluan. Oleh itu, kajian ini telah mendedahkan pelbagai-sebatian, pelbagai-sasaran dan pelbagai-laluan dalam gabungan MO dan GEM terhadap barah pankreas. **Kesimpulan:** Kesimpulannya, kajian *in silico* ini telah mencadangkan bahawa terapi gabungan sebatian bioaktif MO dan GEM meningkatkan kesan secara sinergis dalam rawatan barah pankreas. Walau bagaimanapun, penyelidikan eksperimen lebih lanjut dan aplikasi klinikal selanjutnya diperlukan untuk mengesahkan dapatan ini.

Kata kunci: Moringa oleifera; barah pankreas; sebatian bioaktif; gemcitabine; farmakologi rangkaian

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CHAPTER 1

INTRODUCTION

1.1 Background

According to the World Health Organization, cancer is defined as a disease that occurs in any organ or tissue of the body when the abnormal cells grow and divide uncontrollably beyond the usual boundaries and invade or spread to the other part of the body or organs. Cancer contributes to the second leading cause of deaths worldwide, expected to account for almost 10 million fatalities in 2020 (World Health Organization, 2020). One out of six deaths are due to cancer and represent approximately 9.6 million deaths in 2018. It has been reported that the number of new cancer and cancer-related deaths cases is expected to increase from 16.4 million to 29.5 million per year (World Health Organization, 2018).

Pancreas is the organ that lies transversely in the peritoneum behind the stomach which play an important role in the production of enzymes; trypsin, amylase, lipase that helps in digestion of food and hormones; glucagon and insulin for blood glucose homeostasis. Due to the distinctive anatomical location of pancreas, pancreatic cancer is usually associated with a very poor prognosis as it is often diagnosed at an advanced stage. Pancreatic cancer is one of the most lethal human cancers which appears to be a significant unresolved health issue in the world. Based on GLOBOCAN 2020, the estimated incidence of pancreatic cancer was 495 773 new cases and 466 003 deaths per year making this cancer ranked as 12th most common cancer and 7th leading cause of cancer death in the world, respectively. Through research on epidemiology of pancreatic cancer, Southeast Asia countries recorded much lower rate compared to other countries with the age-standardized incidence rate

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of pancreatic cancer is 2.3 per 100 000 population and 2.2 per 100 000 population for the mortality rate in 2020. Malaysia National Cancer Registry Report, 2019 reported that incidence of pancreatic cancer among Malaysian population is 1.3 per 100 000 population in 2012-2016.

Gemcitabine or also known as dFdC; 2′,2′-difluorodeoxycytidine, a common chemotherapeutic agent that has been used extensively against many kinds of tumor including pancreatic cancer. Gemcitabine has become a golden-standard treatment for locally advanced and metastatic pancreatic cancer since it has been proven to become more superior than fluorouracil (5-FU) in terms of overall survival, performance status and pain control in 1997 (Amrutkar & Gladhaug, 2017). Gemcitabine showed high therapeutic activity with the increasing survival rate in 23.8% of patients treated with gemcitabine compared to patients under influence of 5-FU, 4.8% (Samanta et al., 2019). Although it has been accepted widely, the chemoresistance is one of the major issues associated with this drug. The 5-year survival rate of pancreatic cancer patients with gemcitabine has been reported as low as 2% and 1-year survival rate is only about 17% to 23% (Von Hoff et al., 2013). Currently, studies on drug combination has been well recognized in the cancer research community with the aim to reduce toxicity, minimize the induction of drug resistance as well as to achieve additional therapeutic effect (Hagoel et al., 2019). Thus, the focus had been drawn towards traditional herbal medicine which could be an alternative approach to overcome the limitation of gemcitabine against pancreatic cancer.

Moringa oleifera, a perennial angiosperm tree belongs to the Moringaceae family, commonly known as drumstick or horse-radish tree which can grow as high as 5-10 m and reach a diameter of 20-40 cm at chest height. It is native from Africa and South Asia and most widely cultivated in Northwestern India (Vergara-Jimenez et al.,

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2017). *Moringa oleifera* is grown for its edible teardrop shaped round leaves, small white flower and nutritious drumstick-like fruits which can be used as medicine, food, cosmetics oil and animal forage. Apart from its traditional and nutritional uses, *Moringa oleifera* has demonstrated to possess pharmacological and biological activities such as anti-tumor, antioxidant, anti-microbial, hypoglycemic, hypotensive, hepatoprotective and immunomodulatory (Alhakmani et al., 2013). These could be attributed to the presence of numerous bioactive compounds that have been reported in *Moringa oleifera* studies for its therapeutic effect which are present in significant amount in various components of the plants including flavonoids, phenolic compounds, vitamins, isothiocyanates, saponins and tannins (Vergara-Jimenez et al., 2017). *In vitro* study of *Moringa oleifera* on pancreatic cancer cell, has shown the reduction of 98% Panc-1 cells survival by suppressing the NF-κB signaling pathway (Berkovich et al., 2013).

Many efforts have been made to study the synergistic effects of combination therapy between conventional and traditional herbal medicines. Network pharmacology offers a systematic approach and a novel perspective to present the dynamic relation between drugs, potential targets and associated pathways by constructing the interactions among drug-targets-diseases (Wu et al., 2020). The rapid growth of bioinformatics also provides a powerful platform to study network-based of drug discovery in addressing the complexity of the multi-target mechanism which is more cost-effective drug development approach (Leung et al., 2012).

1.2 Problem statement

The *in vivo* and *in vitro* studies of *Moringa oleifera* is limited to a single pathway in pancreatic cancer treatment which need further investigation. Moreover, the occurrence of gemcitabine tumor resistance significantly restricts the efficiency of

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pancreatic cancer treatment which contributes to the chemotherapy failure (Amrutkar & Gladhaug, 2017). Currently, study on the effectiveness of gemcitabine in combination with *Moringa oleifera* bioactive compounds against pancreatic cancer has not been extensively studied.

1.3 Justification

Network pharmacology, a promising approach toward more cost-effective and time saving in drug discovery, theoretically predict the potential bioactive compound of *Moringa oleifera* and underlying pharmacological mechanism in suppressing pancreatic cancer. Furthermore, *Moringa oleifera*, the Southeast Asia herbal plant that was found to reduce the survival rate of Panc-1 pancreatic cancer cell line, could be a new insight in treating pancreatic cancer. Besides, the research on combination therapy of gemcitabine together with *Moringa oleifera* and their mechanism of action has yet to be explored as it may plays critical role in drug development for pancreatic cancer treatment.

1.4 Objectives

1.4.1 General objective

The aim of this study is to evaluate the potential effect of the combination between *Moringa oleifera* and gemcitabine against pancreatic cancer treatment by the integration of network pharmacology

1.4.2 Specific objectives

To achieve the proposed general objective, the following specific objectives were identified in this research:

i. To determine the bioactive compound of *Moringa oleifera* using the network pharmacology approach

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ii. To screen for *Moringa oleifera* and gemcitabine potential pancreatic cancer-related target genes by using bioinformatics databases

iii. To theoretically evaluate the effects of *Moringa oleifera* and gemcitabine combination therapy against pancreatic cancer through network analysis

1.5 Hypothesis

It can be hypothesized that the combination of bioactive compound in *Moringa oleifera* with gemcitabine might have a synergistic effect in targeting the genes against pancreatic cancer by the integration of network pharmacology.

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CHAPTER 2

LITERATURE REVIEW

2.1 Pancreatic cancer

2.1.1 Type and risk factor

Pancreatic cancer is a gastrointestinal cancer which remains as an intractable disease and leading cause of cancer-related deaths. This disease is usually associated with an extremely poor prognosis as it is often diagnosed at the advanced stage. In the most cases, the cancer has already metastasized by the time of diagnosis due to the rapid development and progression of the tumor with few visible signs and symptoms. Pancreatic cancer can be classified into two types which are pancreatic ductal adenocarcinoma (PDAC) and pancreatic neuroendocrine tumor (PanNET) (Rawla et al., 2019). Pancreatic ductal adenocarcinoma is the most common form of pancreatic cancer which account for more than 80% of the cancer arising in the exocrine gland of the pancreas whereas the minority represent the pancreatic neuroendocrine tumor or also known as islet cell tumor that originate from endocrine tissue of pancreas (Mizrahi et al., 2020).

The risk factors associated with pancreatic cancer development are divided into two types which are modifiable and non-modifiable. Modifiable risk factors are the factors that could be changed according to the behavior pattern including the use of tobacco, obesity and type 2 diabetes (Mizrahi et al., 2020). Study has been demonstrated that people who smoke a pack or more tobacco cigarettes tend to have 5-fold to 6-fold increased risk of pancreatic cancer (Pandol et al., 2012). Furthermore, the development of pancreatic cancer is associated with obesity which has been attributed to the metabolic anomalies such as the resistance of insulin, glucose intolerance and hyperinsulinemia (Sun et al., 2005). Aside from that, diabetes mellitus

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is the third most common modifiable risk factor in pancreatic cancer after the cigarette smoking and obesity (Li, 2012). At the time of diagnosis, 80% of patients with pancreatic cancer develop glucose intolerance impairment due to the peripheral insulin resistance (Wang et al., 2003). Age is one of the major determinants of non-modifiable risk factors in pancreatic cancer. Pancreatic cancer is a condition that mostly affects people above the age of 50 where the peak of incidence is in 7^{th} and 8^{th} decades of life. High mortality rates in elderly patients over the age of 70 have been reported compared to the younger patients (Wang et al., 2020). Besides, pancreatic cancer is more frequently occurring in men than women probably due to the exposure in occupational and environmental factors as well as the lifestyle factors which include high consumption of alcohol and heavy smoking (Rawla et al., 2019). Also, inherited risk factors were contributed for 5-10% of pancreatic cancer cases (Yeo, 2015). Several genes were associated with the hereditary mutation including *STK11, PALB2, BRCA2* and *PRSS1* but not all cases of the diseases could be related to a known gene (Solomon et al., 2012).

2.1.2 Epidemiology

According to the GLOBOCAN 2020, 495,773 new cases and 466,003 deaths of pancreatic cancer in both male and female have been reported, representing 2.6% and 4.7% of all cancers respectively which made pancreatic cancer ranked as the twelfth most common cancer and seventh leading cause of cancer death worldwide (Sung et al., 2021). Despite the significant decrease of mortality rate in other cancers such as stomach, colon, prostate and lung over the past 40 years, the mortality rate of pancreatic cancer remains unchanged or even worsen since it is often associated with a very poor prognosis (Pourshams et al., 2019). Moreover, pancreatic cancer is

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expected to surpass the breast cancer as the third most common cause of cancer death in the future (Mizrahi et al., 2020).

Globally, the estimated age-standardized incidence rate (ASR) of pancreatic cancer is higher compared to the estimated age-standardized mortality rate with approximately 3.9 and 3.7 per 100,000 population, respectively (GLOBOCAN, 2020). In Malaysia, the age-standardized incidence and mortality rate of pancreatic cancer are 3.3 and 3.2 per 100,000 population, ranking fourth after Singapore, Brunei Darussalam and Philippines of South-Eastern Asia countries according to GLOBOCAN 2020. Aside from that, there is a slight increase of the 5-year relative survival rate for pancreatic cancer from 2014 to 2021 which is from 6% to 10%, representing 10 people out of 100 who were alive for five years after being diagnosed with pancreatic cancer (American Cancer Society, 2021). Despite the gradual increment, the number of deaths in pancreatic cancer is still increasing since the majority of the patients are diagnosed at the advanced stage.

2.1.3 Pathophysiology

Pancreatic cancer is often emerged from the pancreatic intraepithelial neoplasia (PanIN), a form of pre-neoplastic lesion which develop in a stepwise manner through the genetic modification to progress into an overt pancreatic ductal adenocarcinoma as shown in Figure 2.1 (McGuigan et al., 2018). However, it can also arise from the large precursor lesions such as mucinous cystic neoplasms (MCNs) or intraductal papillary mucinous neoplasms (IPMNs). PanIN lesion is a non-invasive papillary or flat epithelial neoplasm with less than 5 mm in diameter (Zamboni et al., 2013). It can be categorized into several forms depending on the degree of cytological and architectural atypia from the analysis of histopathological including PanIN-1A, PanIN-1B, PanIN-2 and PanIN-3 (Matsuda, 2019). A two-tiered system has been proposed where PanIN-

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1 and PanIN-2 are considered as low-grade whereas PanIN-3 is classified as high-grade. PanIN-1A and PanIN-1B has a flat and papillary pattern of epithelium, respectively with a minor architectural and cytological atypia while PanIN-2 demonstrate the mild to moderate architectural and cytological atypia and PanIN-3 which is also known as 'carcinoma in situ' is characterized with severe architectural and cytological atypia (Distler et al., 2014). The pancreatic cell will become invasive and spread to the other organ if left untreated which is called as invasive carcinoma.

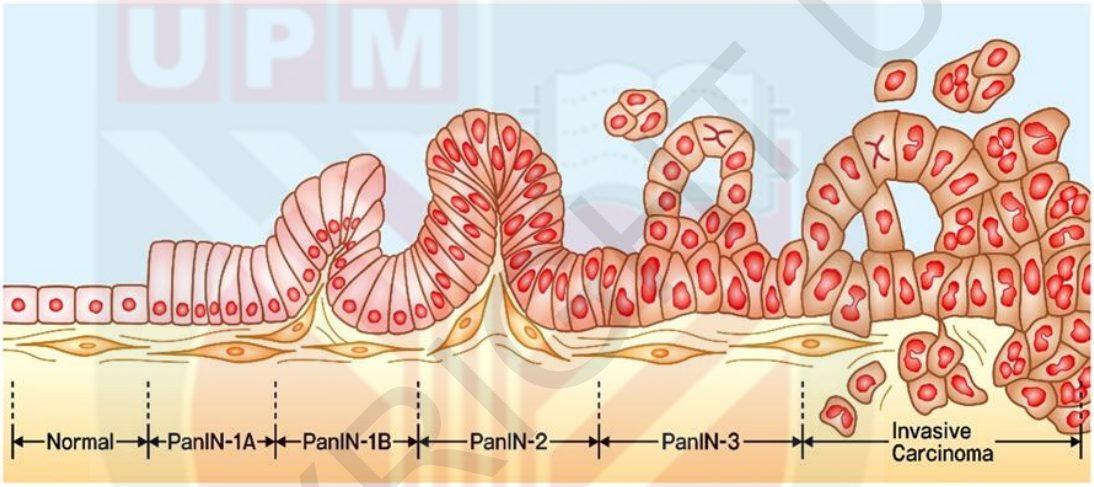

Figure 2.1. The progression of pancreatic cancer (Adapted from Matsuda, 2019).

2.1.4 Molecular pathology

The accumulation of gene mutation in tumor suppressor genes and oncogenes is frequently associated with the development of pancreatic cancer such as the inactivation of tumor suppressor genes such as *TP53, CDKN2A* and *SMAD4* as well as the activation of *KRAS* oncogene (Kamisawa et al., 2016). Approximately, 90% of pancreatic cancer have an activating point mutation in the *KRAS* oncogene located on the human chromosome 12p in the early stage of pancreatic cancer development (Winter et al., 2006). The early detection of *KRAS* mutation can be determined in the duodenal juice, pancreatic juice and stool of the patients (Li et al., 2004). There are

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three major points of mutation detected in codon 12, 13 and 61 which cause the abnormal protein products of *KRAS* with mutation in codon 12 being the most common in pancreatic cancer (Winter et al., 2006). *KRAS* mutation is associated with the formation of PanIN-1 and PanIN-2 which led to the activation of PI3K-AKT and RAS downstream signaling pathway followed by the progression of cell cycle that increased the motility and survival of cancer cells (Mizrahi et al., 2020).

Besides, the inactivation of the cyclin-dependent kinase inhibitor, *CDKN2A* is commonly found in the PanIN-2 development cells (Mizrahi et al., 2020). *CDKN2A* tumor suppressor gene which is encoded by p16 protein is located on the chromosome 9p and inactivated around 95% in pancreatic cancer (Winter et al., 2006). It plays an important role in the cell cycle, apoptosis and cellular senescence regulation (Jiao et al., 2018). It is also known as a negative regulator in the progression of cell cycle which is the transition of G1 to S phase by disrupting the complex development of CDK4/6 and cyclin D (Lin et al., 2020). *CDKN2A* is the most frequently mutated tumor suppressor genes due to the genetic modifications such as mutation, hypermethylation promoter, heterozygosity depletion and homozygous deletion which lead to the downregulation of the gene (Serra & Chetty, 2018). The loss of function in p16 protein will increase the phosphorylation of retinoblastoma (Rb) protein that bind to the E2F transcription factor resulting in cancer cell proliferation (Sun et al., 2020).

The second most frequently inactivated tumor suppressor gene is *TP53* which is located on the chromosome 17p with 50% to 75% of mutation (Winter et al., 2006). This gene encodes the p53 protein. It has the ability to induce the cell cycle arrest, senescence, apoptosis as well as DNA repair. Besides, p53 also induces the apoptosis and proliferative arrest in response to the DNA damage (Mello et al., 2017). It has been reported that the mutation of p53 is usually cause the stable mutated protein expression

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instead of the complete loss of the protein expression (Morton et al., 2010). The *TP53* mutation became a hallmark in the late stage of pancreatic cancer carcinogenesis, indicating the PanIN-3 formation (Mizrahi et al., 2020).

SMAD4 gene or also known as *MADH4* is another tumor suppressor gene located on the human chromosome 18q that is mutated around 50% of pancreatic cancer. Since it has been deleted in the most cases of pancreatic cancer, it is also called as *DPC4*, deleted pancreatic cancer (Ormanns et al., 2017). *SMAD4* gene is mostly involved in the transforming growth factor-β (TGF-β) signaling pathway. The activated TGF-β R1/TGF-β R2 phosphorylate the Smad2/Smad3 proteins which regulate the transcription in the conjunction of Smad4 protein (Shen et al., 2017). In pancreatic cancer patients, the low expression of *SMAD4* is related to the metastasis of lymph nodes and poor prognosis (Martinelli & Lonardo, 2017).

2.1.5 Current treatment

The treatments given for pancreatic cancer patients are vary depending on the stage of the cancer including chemotherapy, radiation therapy and surgery. Surgical resection is the first option of treatment that offers a curative potential with the use of chemotherapy as an adjuvant treatment which has been demonstrated to increase the survival rates (McGuigan et al., 2018). Besides, the neoadjuvant chemotherapy, with or without radiation therapy will be given to the patient in order to improve the surgical margin (Raufi et al., 2019). Pancreatic cancer is categorized into several categories which are resectable, borderline resectable, locally advanced as well as distant metastatic pancreatic cancer (Kamisawa et al., 2016).

In the past few years, the surgical resection of pancreatic cancer cells was associated with an alarmingly high rate of mortality and morbidity. To date, pancreatic cancer surgery can be conducted safely with less than 5% of mortality rate due to the

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training and experience improvement and better management of perioperative. Patients with resectable and borderline resectable tumors can undergo the surgery resection based on the anatomical location of the tumor cell. Pancreaticoduodenectomy or also known as Whipple's procedure is the most common operation in treating pancreatic cancer by removing the head of the pancreas, bile duct, gallbladder, duodenum, jejunum and some part of the stomach (Mizrahi et al., 2020). Besides, distal pancreatectomy which is also associated with splenectomy is used to treat the tumors that are located in the body or tail of the pancreas while a total pancreatectomy is the surgical procedure that involve the removal of the entire pancreas (Mizrahi et al., 2020).

Adjuvant chemotherapy is given to the patient after the primary treatment such as surgery in order to kill the remaining cancer cells. Recent studies showed that the administration of adjuvant gemcitabine for six cycles improved the survival of the disease compared to no adjuvant therapy following surgery (Kamisawa et al., 2016). However, 80% of pancreatic cancer patients that undergo surgical resection developed the tumor recurrence despite the improvement in the surgical procedure and advanced adjuvant treatments (Moletta et al., 2019). The use of neoadjuvant treatment also has been reported in the resectable and borderline resectable pancreatic cancer patients. In the recent retrospective study, the increase of overall survival rate was exhibited in the resected patient that received neoadjuvant treatment compared to the patient who received the adjuvant treatment (Mizrahi et al., 2020).

Apart from that, patients with locally advanced diseases are usually caused by the involvement of the extensive vascular system that prevents surgical resection (Mizrahi et al., 2020). Systemic chemotherapy such as FOLFIRINOX or gemcitabine plus nab-paclitaxel is the option of the treatment for this kind of pancreatic cancer as

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well as distant metastasis. Neoadjuvant chemoradiation therapy in locally advanced pancreatic cancer patients has been extensively studied. However, its role against locally advanced pancreatic cancer became a controversial issue since studies reported contradictory findings (Mizrahi et al., 2020).

2.2 Gemcitabine

2.2.1 Introduction

Nucleoside analogue-derived drugs are often used as cancer and antiviral therapy which include gemcitabine, a cytidine analogue that possesses the broad-spectrum of anticancer activity (Zhong et al., 2020). Gemcitabine was initially studied as an antiviral agent before being developed into the anticancer drug due to its remarkable antitumoral effect *in vitro* and *in vivo*. In 1997, the effectiveness of gemcitabine in the advanced stage of pancreatic cancer was proven which it became the preferred treatment for metastatic pancreatic cancer (Ergun et al., 2018).

Gemcitabine acts by preventing the cancer cells from dividing and inhibits the synthesis of DNA which leads to the cell deaths or apoptosis as shown in Figure 2.2. It is transported to the cells via five nucleoside transporters such as hENT1, hENT2, hCNT1, hCNT2 and hCNT3 (Amrutkar & Gladhaug, 2017). In the cells, gemcitabine (dFdC) is phosphorylated by deoxycytidine kinase (dCK) into gemcitabine monophosphate (dFdCMP). This conversion is considered as the rate-limiting step. Subsequently, gemcitabine diphosphate (dFdCDP) and gemcitabine triphosphate (dFdCTP) are phosphorylated by the nucleoside monophosphate kinase (NMPK or UMP/CMP) and nucleoside diphosphate kinase (NDPK), respectively (Zeng et al., 2019). dFdCDP inhibits ribonucleoside-diphosphate reductase (RR), a key enzyme which responsible for the conversion of ribonucleotides to deoxyribonucleotides in the regulation of DNA synthesis (Pereira et al., 2004). RR converts cytidine diphosphate

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(CDP) to deoxycytidine diphosphate (dCDP) and the inhibitory action causes a decreased number of competitive deoxycytidine triphosphate (dCTP) pool cells which then allow dFdCTP to bind to DNA. On the other hand, dFdCTP causes the termination of chain and DNA synthesis suppression in the nucleus when it is being incorporated into DNA resulting in the cell death by apoptosis (Amrutkar & Gladhaug, 2017). However, rapid deamination caused by cytidine deaminase (CDA) inactivates the majority of the administered gemcitabine that lead to high level of less active metabolite of gemcitabine, 2',2'-difluorodeoxyuridine (dFdU). The inactivation of dFdCMP by deoxycytidylate deaminase (DCTD) into difluorouridine monophosphate (dFdUMP) which involves a proper amount of active dCTP, is suppressed by dFdCTP and intracellular reduction of dCTP (Amrutkar & Gladhaug, 2017).

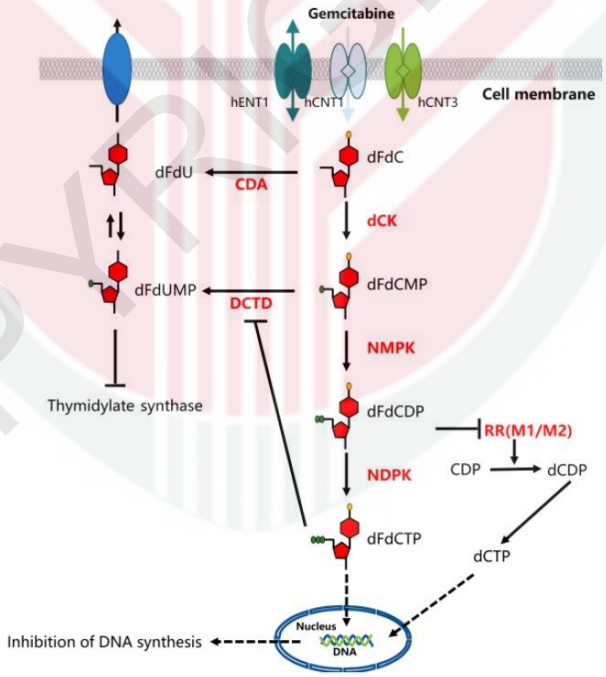

Figure 2.2. The mechanism of action of gemcitabine (Adapted from Zeng et al., 2019).

2.2.2 Combination based therapy with traditional herbal medicines

Ergun et al. (2018) reported that gemcitabine monotherapy has a short overall median survival which is 5 to 7 months in pancreatic cancer which led to the further

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studies of gemcitabine combination therapy that could enhance its therapeutic effects. Study on the combination of gemcitabine with escin has been conducted to evaluate the effectiveness of the combination drug. Escin is isolated from horse chestnut seed which is commonly used as analgesic and antipyretic agent. It has been found that escin improved the effectiveness of gemcitabine by inactivating the NF-κB signaling pathway and inhibiting Bcl-2, c-Myc, survivin, cyclin D1, COX-2 and Bcl-xL as well as the caspase-3 activation against pancreatic cancer (Wang et al., 2012). Besides, *Clinacanthus nutans* extract together with gemcitabine had a synergistic effect in inducing the apoptotic nucleosomes of pancreatic cancer cells, AsPC1, BxPC-3 and SW1990 cell lines by the downregulation of XIAP, Bcl-2 and cIAP-2 as well as Bax upregulation (Hii et al., 2019). Huang et al. (2010) also found that the combination-based therapy of gemcitabine with gum mastic, a natural resin extracted from the *Pistacia lentiscus* tree induce potent apoptosis activity by inhibiting the NF-κB pathway on the proliferation of BxPC-3 and COLO 357 human pancreatic cancer cell lines (Huang et al., 2010).

2.3 Moringa oleifera

2.3.1 Traditional uses

Moringa oleifera (MO) has been known and used widely as traditional herbal medicines in both traditional medical systems including Ayurveda and Traditional Chinese Medicines (TCM) (Flora & Pachauri, 2011). Every part of the tree including flowers, leaves, barks, roots, seeds and fruits can be used either as medicinal or nutritional purposes due to the high value of nutrition. In Ayurveda, MO is used as a pain relief and worm expulsion as well as for wound healing (Biswas et al., 2019). The leaves of MO are rubbed on the flat stone with water being gradually added and its paste will be used for external application (Rathi et al., 2004). From the previous study,

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the seed and flower of MO were used in chickenpox and smallpox treatment which aid in the production of immunity against those diseases (Biswas et al., 2019). MO has also been used to treat AIDS and HIV-related secondary infections in Africa. It has been reported in the previous study that the use of MO as the agent of diuretic can treat several kinds of disease including kidney stones, prostatitis, water retention, scalding urine and obesity (Adeyemi & Elebiyo, 2014). According to the TCM, the herbal plant is classified into its taste and temperature where MO is considered to have cold and bitter taste which exhibited the antihypertensive properties and promote defecation (Meireles et al., 2020). In addition to the medicinal properties, MO seeds can also be used for oil extraction that substitute the olive oil for cooking, machine lubricant, biodiesel and cosmetics (Matic et al., 2018).

2.3.2 Phytochemical properties

Moringa oleifera consist of diverse phytochemical constituents as each part of this herbal plant contributes to several types of chemical compounds. It is believed that the wide range of MO phytochemical constituents were associated with its numerous pharmacological activities. There are several bioactive compounds that can be found in MO where it can be classified as flavonoids, glucosinolates, isothiocyanates, phenolic acids, alkaloids, vitamins, saponins and tannins (Leone et al., 2015).

Flavonoids are ubiquitous and can be categorized as a polyphenols sub-group with benzo-γ-pyrone structure. The high consumption of flavonoids has been shown to protect against a variety of degenerative conditions such as cancer and cardiovascular and infectious diseases including viral and bacterial infections. In MO leaves, kaempferol, quercetin and myricetin are considered as the major flavonoids while some other flavonoids such as apigenin, luteolin, genistein and daidzein can also

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be found in undetectable concentration (Leone et al., 2015). Quercetin and kaempferol can also be found in the flower and seed of MO (Huang et al., 2020).

Besides, phenolic acids are another sub-group of polyphenols derived from hydroxycinnamic acid and hydrobenzoic acid that is naturally found in plants. There is abundance of phenolic acids present in the MO leaves such as gallic acids, caffeic acids, chlorogenic acids, ferulic acids and p-coumaric acids. These compounds also present in the MO seed and are synthesized to study the anticancer, anti-inflammatory and antioxidant activities, particularly (Huang et al., 2020; Leone et al., 2015).

Glucosinolates are also known as secondary metabolites in plants (Leone et al., 2015). Glucosinolate is broken down into isothiocyanates by the action of myrosinase enzyme through the hydrolysis activity (Angelino et al., 2015). Cruciferous vegetables such as cabbage, broccoli and radish are the major source of these compounds. 4-O-(α-l-rhamnopyranosyloxy)-benzyl glucosinolate or known as glucomoringin are the most abundant glucosinolate present in MO leaves (Rani et al., 2018). Glucosinolates and isothiocyanates were reported to play a role as disease prevention and health promotion (Leone et al., 2015).

Other phytochemicals that are present in MO are vitamins. There are several types of vitamins found in MO including vitamin A, B and E. Carotenoids such as β-carotene present in a huge amount in fresh leaves of MO compared to pumpkin, carrot and apricot which serves as pro-vitamin A. Riboflavin, thiamine and niacin which are among of the vitamin B were found in MO leaves that function as a cofactor in energy production and nutrient metabolism. Vitamin E which acts as antioxidant is present in the MO leaves including α-tocopherol particularly (Leone et al., 2015).

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Last but not least, alkaloids are one of the naturally occurring compounds with basic nitrogen atoms. Rani et al. (2018) found two new alkaloid glycosides that were isolated in the leaves of MO which are marumoside A and marumoside B. Besides, saponin in MO have the anticancer properties despite the presence of hemolytic side effect. Tannins, the water-soluble compounds also demonstrated several therapeutic effects including anticancer and anti-inflammatory (Leone et al., 2015).

2.3.3 Pharmacological activities

Moringa oleifera is the herb with a wide range of pharmacological properties including anticancer, antioxidant, antidiabetic, anti-inflammatory and antimicrobial activities. Studies have been focused on the MO leaves extract to evaluate their anticancer activity compared to the other part of MO. It has been shown that the formation of colony and cell motility of (HCT-8) colorectal and (MDA-MB-231) breast cancer cell line were reduced upon the treatment with MO extract as well as detection of high apoptosis, low survival of cell and enrichment in the G2/M phase with MO leaves and bark extracts (Al-Asmari et al., 2015). In pancreatic cancer, Berkovich et al. (2013) tested the anticancer activity of MO leaves extract on three different cell lines which are Panc-1, COLO-357 and p34. Based on the XTT-based colorimetric assay, 2mg/mL of MO extract has reduced the 98% of Panc-1 cell survival by the downregulation of NF-κB signaling pathway.

Furthermore, MO also possesses some of the antioxidant activities. In the recent studies, the modulatory action of MO leaves extract towards the cytotoxicity and oxidative damage induced by H_2O_2 in the HeLa-derived KB cell line has been explored. Results of the single cell electrophoresis revealed that the DNA damage in KB cells decreased significantly when the cells were treated with both tender and mature leaves of MO extracts compared to the untreated cell (Khor et al., 2018).

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Next, the *in vivo* study of antidiabetic activity was performed to evaluate the methanol extract of MO pods in the diabetic albino rats induced by streptozotocin (STZ). The diabetic rats were treated for 21 days by given 150 or 300 mg/kg of MO extract and the biochemical parameters changes in the serum and tissue of pancreatic cancer were observed. After the treatment with MO extract, the diabetes progression as well as nitric oxide and glucose serum was substantially decreased while the serum insulin and protein level were significantly increased in both doses of the extract (Gupta et al., 2012). Other studies on STZ-induced diabetic male rats also showed the reduction of antioxidant enzymes in the serum and kidney tissue with the high level of *IL6* and lipid peroxide (Al-Malki & El Rabey, 2015).

Apart from that, the anti-inflammatory study of rat hind paw edema induced by carrageenan was conducted for 10 days by treating the rat with three different solution and doses, 5 ml/kg/day of normal saline for group 1, 0.5 mg/kg/day of dexamethasone for group 2 and 200 mg/kg/day of MO extract for group 3. Finding of this study revealed that the 200 mg/kg of aqueous extract of MO possesses the anti-inflammatory action by reducing the inflammation effect on the hind paw of the rat (Mittal et al., 2017).

Lastly, antimicrobial activity was higher in the ethanol extract of MO compared to the cold extract in treating the abscess in camel which is known as the camel infections that is caused by the pyogenic bacteria including *Streptococci* spp., *Klebsiella pneumoniae, Staphylococci* spp. and *Citrobacter* spp. (Fouad et al., 2019). Also, it has been reported that the extract of MO leaves inhibits the *Staphylococcus epidermidis* in the concentration of 8% b/v with the average inhibition zone of 14mm compared to the 2% and 4% b/v (Ervianingsih et al., 2019).

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2.4 Network-based pharmacology

Bioinformatics is a comparatively recent approach that encompasses the classical bioinformatics and biology systems (Oulas et al., 2017). Over time, various tools have been built to analyze huge amount of data as bioinformatics is a large and complex multidisciplinary research field. It has been exhibited in the previous study that bioinformatic analysis is one of the useful approaches for determining the mechanism of tumorigenesis, development and treatment of cancer (Shen et al., 2019).

Network pharmacology is the bioinformatic approach that combines both experiment and computation systems for a better understanding of drug activity at multiple levels ranging from the molecular and cellular stages to the tissue and organism stages (Berger & Iyengar, 2009). It is also another strategy to discover a new lead and target of a drug as well as to repurpose the current drug for various therapeutic treatments without the unbiased analysis of the potential target (Chandran et al., 2017). This network pharmacology study has first been proposed by Andrew L. Hopkins which incorporates the bioinformatics, systems biology and poly-pharmacology (Shen et al., 2019). The association of multiple genes and functional proteins are involved in the most complex disease. Network pharmacology not only explores the "multi-components" and "multi-targets" but also recognizes the drug-gene-disease interactions which demonstrate the molecular level of drug therapeutic mechanisms (Zhou et al., 2020). It has been reported that this approach increased the efficacy of the drug as well as reduced the side effects of the drug and drug development cost (Zhang et al., 2013). In recent years, network pharmacology research has greatly benefited by the availability of a range of relevant databases and technologies such as compounds and drugs information databases (STITCH, ChEMBL and Drugbank databases), target interaction databases (MINT, HPRD and IntAct databases) as well as genes and

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diseases association databases (GAD and OMIM databases) (Zhang et al., 2019). In network pharmacology, there are several steps involved to theoretically evaluate the effectiveness on the combination of drug between herbal medicines and conventional drugs including the identification of bioactive compounds and target genes, protein-protein interaction, target enrichment analysis and network construction (Figure 2.3).

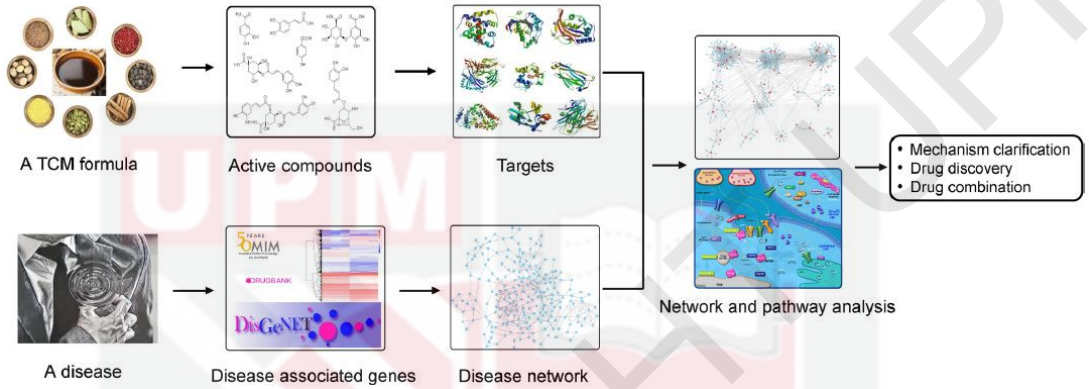

Figure 2.3. The general workflow of network pharmacology approach in Traditional Chinese Medicines formula (Adapted from Zhao et al., 2019).

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CHAPTER 3

METHODOLOGY

3.1 Identification of chemical compounds of MO

Identification of chemical compound of MO were performed by using Traditional Chinese Medicine Systems Pharmacology, TCMSP (https://www.tcmspw.com/tcmsp.php, accessed on 2 December 2020) (Ru et al., 2014), a unique Chinese herbal medicine pharmacology database system which integrate the association between drugs, targets and diseases network, and Traditional Chinese Medicine Integrated Database, TCMID (http://bidd.group/TCMID/, accessed on 2 December 2020) (Xue et al., 2013). The Latin name of MO was searched in these two databases to retrieve the composition of chemical and pharmacokinetic properties of each chemical compound. However, the constituents of MO cannot be found in the databases.

Alternatively, studies related to MO were searched through the electronic literature database, PubMed, (https://pubmed.ncbi.nlm.nih.gov/, accessed on 7 December 2020) (Fiorini et al., 2017), to determine the biochemical compounds. Huang et al., 2020 studied the effect of anti-insulin resistance of MO and listed the compounds found in MO in their article.

3.2 The *in silico* screening of bioactive compounds in MO

The absorption, distribution, metabolism and excretion (ADME) properties have been regarded as essential indicators for orally administered herbal medicine. The evaluation of three parameters including the oral bioavailability (OB), drug-likeness (DL) and Caco-2 permeability were applied to predict the potential bioactive compounds of MO. Oral bioavailability (OB) is referred to the fraction of orally administered dosage that enters the site of action capable of generating

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pharmacological activities (Huang et al., 2020). OB is the most crucial parameter in the ADME processes of compound screening which has the potential to be further developed into drugs (Li et al., 2012). Besides, drug-likeness (DL) is the identification of pharmacokinetic and pharmaceutical properties of the compound based on the Lipinski's rule of five including the molecular weight, octanol-water partition coefficient log P, hydrogen bond donors and hydrogen bond acceptors (Huang et al., 2020). Caco-2 permeability is the ability of a drug to be absorbed into the intestine through the cell which is derived from human epithelial colorectal adenocarcinoma cell line. The three parameters were evaluated using *in silico* integrative ADME model through the TCMSP database with the threshold of OB ≥ 30%, DL ≥ 0.18 and Caco-2 permeability ≥ -0.4 (Lee & Oh, 2020). Refer Appendix I for detailed steps on screening the bioactive compounds of MO. Compounds listed in Huang et al., 2020 that met the criteria of ADME properties were considered as bioactive compounds. The bioavailability details of some MO compounds, which cannot be found in the TCMSP database were searched through online published literature using PubMed.

3.3 Target identification

3.3.1 Target genes prediction of MO bioactive compounds

Drugbank (https://go.drugbank.com/, accessed on 17 December 2020) (Wishart et al., 2018), The Drug Gene Interaction Database, DgIdb (https://www.dgidb.org/, accessed on 17 December 2020) (Freshour et al., 2020) & Comparative Toxicogenomics Database, CTD (http://ctdbase.org/, accessed on 17 December 2020) (Davis et al., 2021) databases were used to retrieve the target genes of MO. All the target genes were limited to "Homo sapiens" only and duplicated target genes were removed from the list. Refer Appendix II for detailed steps on predicting the target genes of MO.

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3.3.2 Prediction of GEM target genes

Several databases were employed for GEM target genes prediction including Drugbank (https://go.drugbank.com/, accessed on 17 December 2020) (Wishart et al., 2018), The Drug Gene Interaction Database, DgIdb (https://www.dgidb.org/, accessed on 17 December 2020) (Freshour et al., 2020) & Comparative Toxicogenomics Database, CTD (http://ctdbase.org/, accessed on 17 December 2020) (Davis et al., 2021) databases. Genes that are belong to "Homo sapiens" were included in the list and duplicated target gene were eliminated. Refer Appendix III for detailed steps on predicting the gemcitabine target genes.

3.3.3 Prediction of pancreatic cancer target genes

The target genes related to pancreatic cancer were derived from two databases which are Online Mendelian Inheritance in Man, OMIM (https://www.omim.org/, accessed on 17 December 2020) (Amberger et al., 2015) and Malacards (http://www.malacards.org/, accessed on 17 December 2020) (Rappaport et al., 2013) with keyword "pancreatic cancer". Refer Appendix IV for detailed steps on predicting the pancreatic cancer target genes.

3.4 Screening potential targets of MO and GEM

All the predicted target genes for MO, GEM and pancreatic cancer were imported into the Bioinformatics and Evolutionary Genomics (http://bioinformatics.psb.ugent.be/webtools/Venn/, accessed on 17 December 2020) to identify the pancreatic cancer-related target genes of MO and GEM. The Venn diagram constructed from the tool represent the intersection of potential target genes between drug and disease. Refer Appendix V for detailed steps on screening the potential targets between MO and GEM.

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3.5 Protein-protein interaction (PPI) network analysis

Protein-protein interaction (PPI) network was constructed via The Search Tool for the Retrieval of Interacting Genes, STRING database, version 11.0 (https://string-db.org/, accessed on 18 December 2020) (Szklarczyk et al., 2019) by inputting the target genes of MO and GEM against pancreatic cancer for a better understanding of the protein interactions. The PPI networks were set as "Homo sapiens" organism, highest confidence (0.900) for the minimum required interaction score and exclude the disconnected node in the network. Refer Appendix VI for detailed steps on constructing the protein-protein interaction network.

3.6 Identification of hub genes

The PPI network with default settings were imported into Cytoscape software, version 3.8.2 (https://cytoscape.org/) (Shannon et al., 2003), an open source software tool for visualizing and integrating complex interaction network with any kind of attribute data (Wan et al., 2019) for the identification of hub genes. The hub genes were identified through Cytoscape plug-in cytoHubba by first selecting the top 10 genes under the, i.e., "Degree", "Closeness" and "Betweenness" parameters to calculate the topological features of each node in the network. Degree can be defined as the number of edges connected to a node (Yin et al., 2020). Closeness is considered as the proximity of a node to the other nodes (Tao et al., 2020). Betweenness is referred to the measurement of nodes according to the shortest paths (Bao et al., 2020). The shared top ten hub genes by the three parameters were determined using the Venn diagram tool. Refer Appendix VII for detailed steps on identifying the hub genes.

3.7 Network construction

The PPI network with default settings were used for network construction. Compound-target-pathway network construction was established by using Cytoscape

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software, version 3.8.2 to better demonstrate the interaction and pharmacological mechanism of MO combined with GEM against pancreatic cancer. Two networks including compound-target network and target-pathway network were built separately prior to merging into a compound-target-pathway network. Compound-target (C-T) network was constructed to show the interaction between the combination of MO and GEM with their respective target genes. Refer Appendix VIII for detailed steps on constructing the compound-target network. Besides, target-pathway (T-P) network was constructed to show the interaction between the combination of MO and GEM with the pathways involved. Refer Appendix IX for detailed steps on constructing the target-pathway network). In the C-T network, each compound node were analyzed and ranked according to their "degree" value and nodes with more than average node of degree were considered as hub compounds.

3.8 Gene ontology and pathway enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery, DAVID, version 6.8 (https://david.ncifcrf.gov/home.jsp, accessed on 27 January 2021) (Dennis et al., 2003), an online software that offers comprehensive data for high-throughput gene functional analysis in the context of clarifying the biological characteristics, was used to perform the Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis (Liang et al., 2020). The enrichment analysis was conducted to reveal the underlying mechanism of the combination between MO and GEM against pancreatic cancer through the biological process, cellular components, molecular functions and key signaling pathway. All the target genes were inputted and selected as "Homo sapiens" species. The Expression Analysis Systematic Explorer (EASE) scores ≤ 0.5 and Count

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≥ 2 were set according to the default settings. Refer Appendix X for detailed steps on identifying the gene ontology and pathway enrichment analysis.

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CHAPTER 4

RESULTS

4.1 Identification and screening of MO compound

According to the literature of Huang studies, a total of 32 compounds have been identified in MO (Table 4.1). However, only 7 compounds were found to be listed in the TCMSP database which are catechin, epicatechin, quercetin, kaempferol, caffeic acid, p-coumaric acid and ferulic acid. Among those, all the 7 compounds passed one of the drug screening criteria which is OB ≥ 30% but only 4 compounds which are catechin, epicatechin, quercetin and kaempferol that met the three requirement of the drug screening which are OB ≥ 30%, DL ≥ 0.18 and Caco-2 permeability ≥ -0.4 (Table 4.2). Therefore, catechin, epicatechin, quercetin and kaempferol were selected as the bioactive compound that were used for further analyses.

The screening of the remaining 25 compounds of MO were conducted using DgIdb, CTD and Drugbank databases to search for the target genes. Target genes were found only for three compounds out of the 25 compounds i.e., glucomoringin, glucoraphanin and moringinine. The bioavailability of these compounds was determined through literature search since these compounds were not found in the TCMSP database. Glucomoringin and glucoraphanin were reported to have a good bioavailability while the solubility of moringinine can be improved by the formation of benzylamine salts with benzoic acid derivative (Ali et al., 2016; Fahey et al., 2019; Mathiron et al., 2018; Michl et al., 2016; Parshad et al., 2002, 2004; Thinh Nguyen et al., 2020). These 3 compounds were also regarded as bioactive compounds as they were reported to exhibit anticancer activity in several types of cancers (Ali et al., 2016; Almuhayawi et al., 2020; Rajan et al., 2016).

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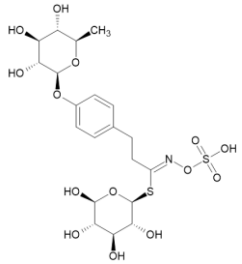
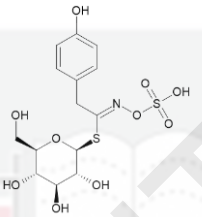
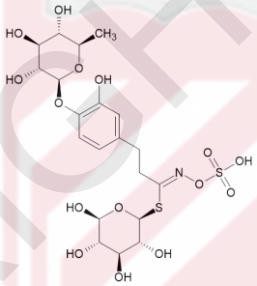
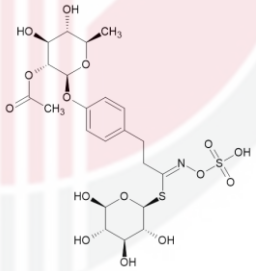
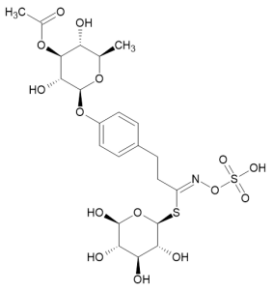
Table 4.1. The bioactive compounds in MO

Compound	Chemical structure	Class/Group
Glucomoringin		Benzyl glucosinolate
Glucosinalbin		Alkyl glucosinolate
3-hydroxyglucomoringin		Isothiocyanate
4-(2'-acetyl-α-L-rhamnosyloxy)-benzyl isothiocyanate		Benzyl isothiocyanate
4-(3'-acetyl-α-L-rhamnosyloxy)-benzyl isothiocyanate		Benzyl isothiocyanate

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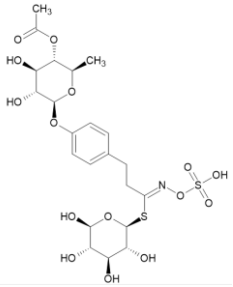
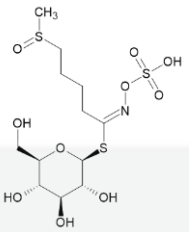
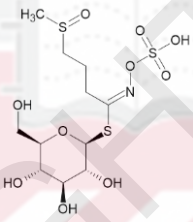
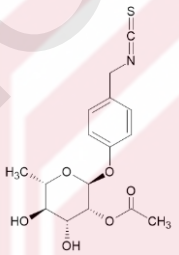
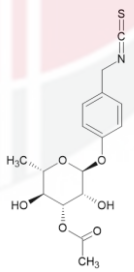
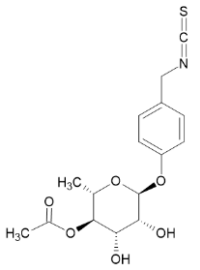
4-(4'-acetyl-α-L-rhamnosyloxy)-benzyl isothiocyanate		Benzyl isothiocyanate
Glucoraphanin		Alkyl glucosinolate
Glucoiberin		Alkyl glucosinolate
4-[(2'-O-acetyl-α-L-rhamnosyloxy)benzyl] isothiocyanate		Benzyl isothiocyanate
4-[(3'-O-acetyl-α-L-rhamnosyloxy)benzyl] isothiocyanate		Benzyl isothiocyanate
4-[(4'-O-acetyl-α-L-rhamnosyloxy)benzyl] isothiocyanate		Benzyl isothiocyanate

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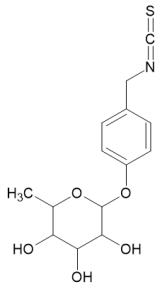
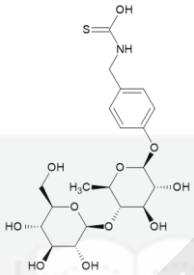
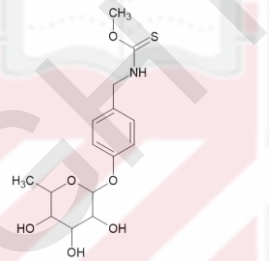
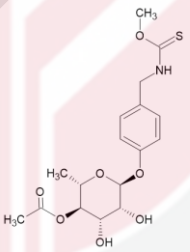
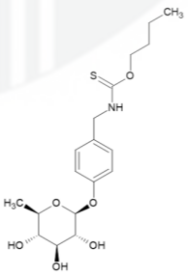
Moringin		Isothiocyanate
4-[β-D-glucopyranosyl (1→ 4)-α-L-rhamnopyranosyl]benzyl thiocarboxamide		Benzylamine
Niazinin A		Phenolic glycoside
Niazicin A		Phenolic glycoside
O-butyl-4-[(α-L-rhamnopyranosyloxy)-benzyl] thiocarbamate (E)		Benzylamine

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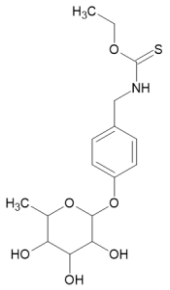
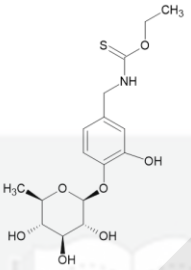
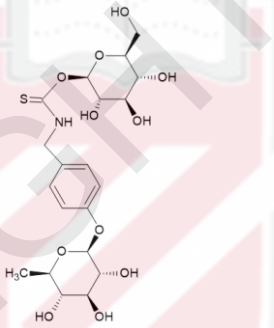
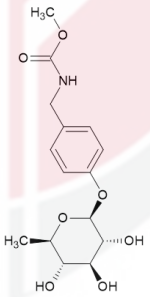
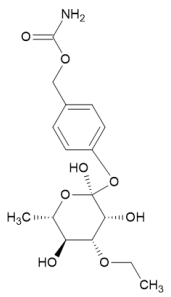
Niazimicin		Phenolic glycoside
O-ethyl-4-[(α-L-rhamnopyranosyloxy)-3-hydroxybenzyl] thiocarbamate (E)		Benzylamine
N-[4-(β-L-rhamnopyranosyl)-benzyl]-1-O-α-D-glucopyranosyl-thiocarboxamide		Benzylamine
Methyl N-[4-[(α-L-rhamnopyranosyl)benzyl]] carbamate		Benzylamine
O-ethyl-4-[(α-L-rhamnosyloxy)-benzyl] carbamate		Benzylamine

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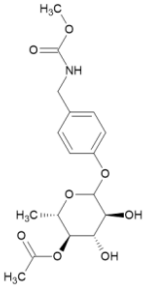
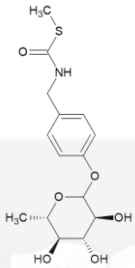
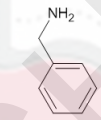
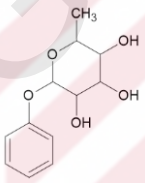
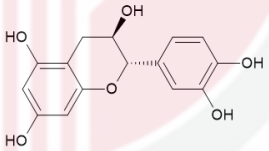
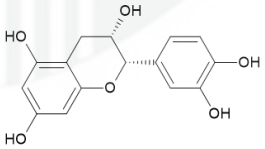
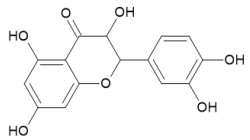
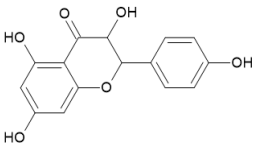
Methyl N-[4-[(4'-O-acetyl-α-L-rhamnopyranosyl)benzyl]] carbamate		Benzylamine
S-methyl-N-[4-[(α-L-rhamnosyloxy)benzyl]] thiocarbamate		Benzylamine
Moringinine		Benzylamine
1-O-phenyl-α-L-rhamnopyranoside		Phenylpropanoid
Catechin		Flavonoid
Epicatechin		Flavonoid
Quercetin		Flavonoid
Kaempferol		Flavonoid

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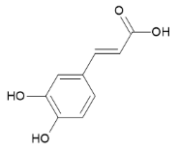
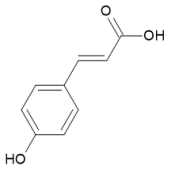
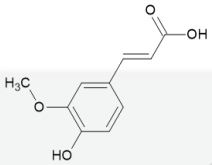
Caffeic acid		Phenolic compound
P-Coumaric acid		Phenolic compound
Ferulic acid		Benzyl glucosinolate

Table 4.2. The ADME properties of MO compounds found in Traditional Chinese Medicine Systems Pharmacology (TCMSP).

Compound	**Oral bioavailability OB (≥ 30%)**	**Drug-likeness DL (≥ 0.18)**	**Caco-2 permeability (≥ -0.4)**
*Catechin	54.83	0.24	-0.03
*Epicatechin	48.96	0.24	0.02
*Quercetin	46.43	0.28	0.05
*Kaempferol	41.88	0.24	0.26
Caffeic acid	54.97	0.05	0.27
P-Coumaric acid	43.29	0.04	0.46
Ferulic acid	39.56	0.06	0.47

*Compounds that met the three criterias of the drug screening

4.2 Target genes prediction of MO, GEM and pancreatic cancer

In MO, a total of 1092 potential pancreatic cancer target genes of the 7 bioactive compounds were found in Drugbank, DgIdb and CTD databases. There are

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352 and 421 target genes for GEM and pancreatic cancer respectively that were retrieved from several databases.

4.3 Screening of potential pancreatic cancer-related target genes of MO and GEM

The potential pancreatic cancer-related target genes of MO and GEM were represented by the Venn diagram (Figure 4.1). The diagram revealed 70 target genes that were overlapped between MO and GEM (Figure 4.1a). Also, 89 GEM-intersection (Figure 4.1b), 81 MO-intersection (Figure 4.1c), 137 MO+GEM-intersection (Figure 4.1d) target genes and 33 shared biotargets of MO and GEM against pancreatic cancer were identified using the online database (Figure 4.1d).

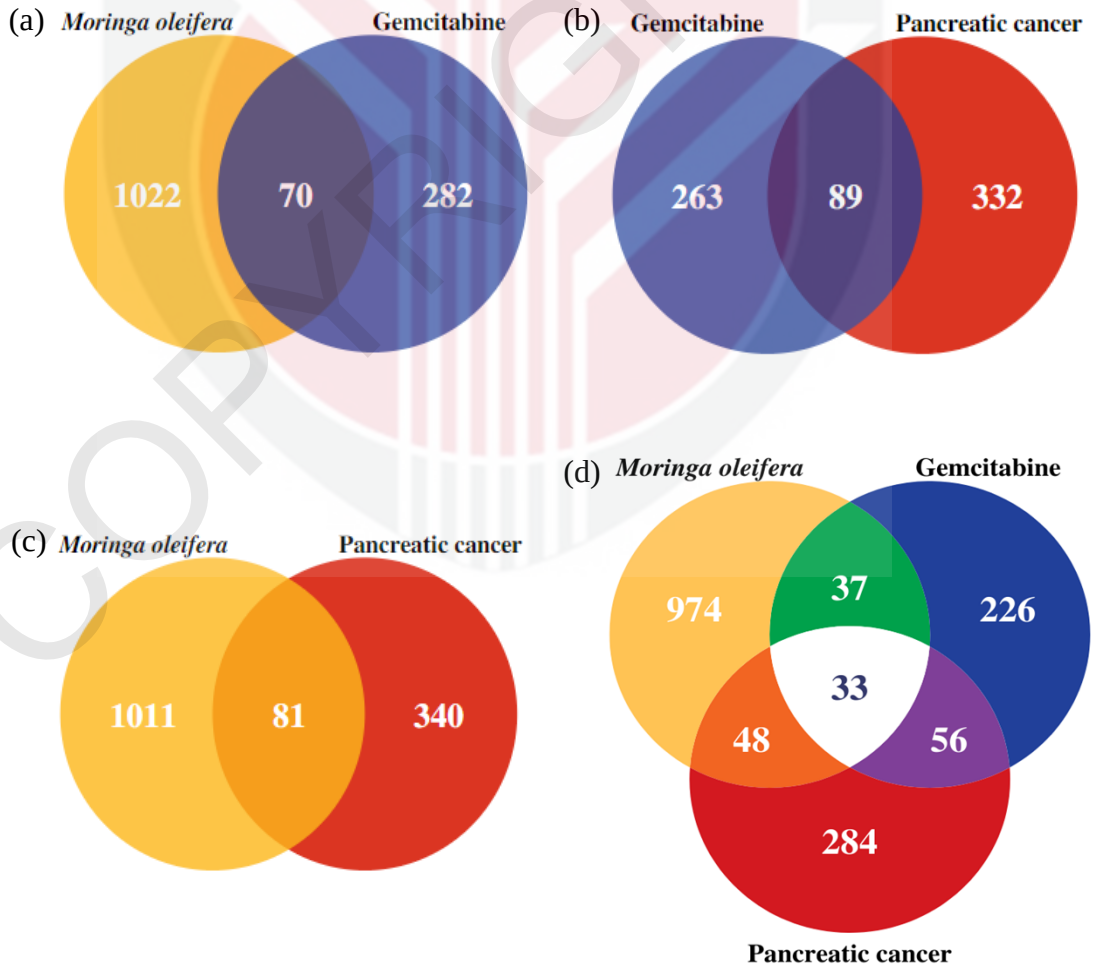

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Figure 4.1. The relation of target genes via Venn diagram between 7 compounds of MO and GEM against pancreatic cancer. (a) Venn diagram showed 70 target genes between MO and GEM-intersection. (b) Venn diagram showed 89 target genes in GEM-intersection against pancreatic cancer. (c) Venn diagram showed 81 target genes in MO-intersection against pancreatic cancer. (d) Venn diagram showed 137 target genes in MO+GEM-intersection (orange, white and purple) and 33 target genes in shared biotargets-intersection (white) against pancreatic cancer.

4.4 PPI network

In this study we investigated 4 intersections. The PPI network of the MO+GEM-intersection (Figure 4.2a), shared biotargets-intersection (Figure 4.2b), MO-intersection (Figure 4.2c) and GEM-intersection (Figure 4.2d) targets against pancreatic cancer were constructed using STRING database. There are 136 nodes with one disconnected node (MIR24-2) and 878 edges of MO+GEM-intersection, 33 nodes and 122 edges of shared biotargets-intersection, 81 nodes and 373 edges of MO-intersection and 88 nodes with one disconnected node (MIR24-2) and 425 edges of GEM-intersection targets against pancreatic cancer. The networks showed that these target genes have complex interaction between each other.

Several potential pancreatic cancer-related target genes of MO and GEM also have been identified in the network and were considered as the hub genes based on the degree, closeness and betweenness values. All the hub genes for each intersection were shown in Figure 4.3. *TP53, AKT1, VEGFA* and *CCND1* were identified as the hub genes that overlapped between the four intersection networks. Shared biotargets-intersection has two hub genes, *CASP3* and *BCL2L1*, that are not targeted by GEM or MO alone, while *STAT3* was the only hub gene that is untargeted by any other intersections except GEM-intersection. *IL6* together with *TP53, AKT1, VEGFA* and *CCND1* were found to be the hub genes that overlapped between MO+GEM- and MO-intersections. *EGFR* and *MYC* together with *TP53, AKT1, VEGFA* and *CCND1* were

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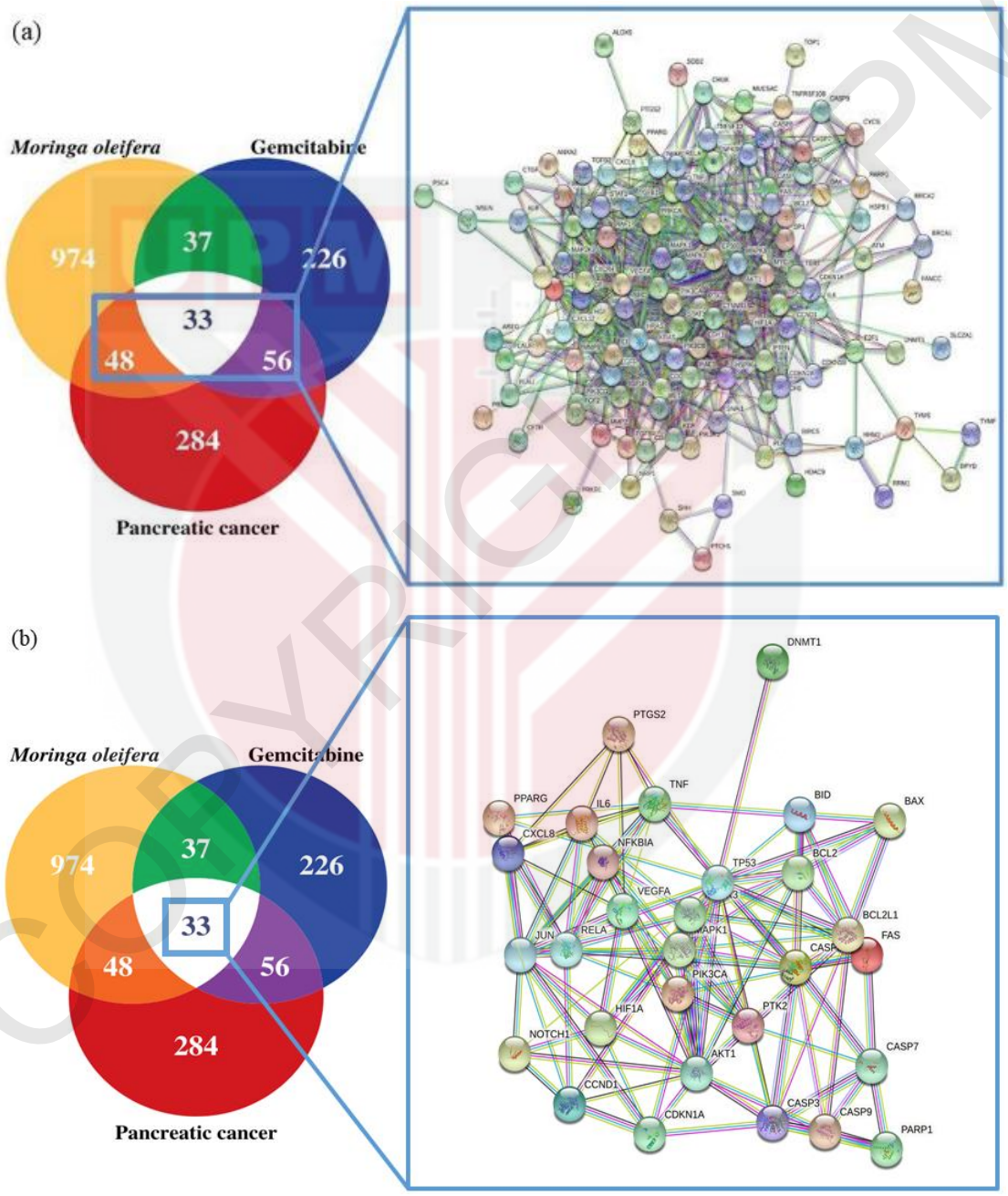
identified as the hub genes that overlapped between MO+GEM- and GEM-intersections. Four hub genes which are *MAPK3*, *JUN*, *MAPK1* and *TNF* were overlapped between MO- and shared biotargets-intersections against pancreatic cancer.



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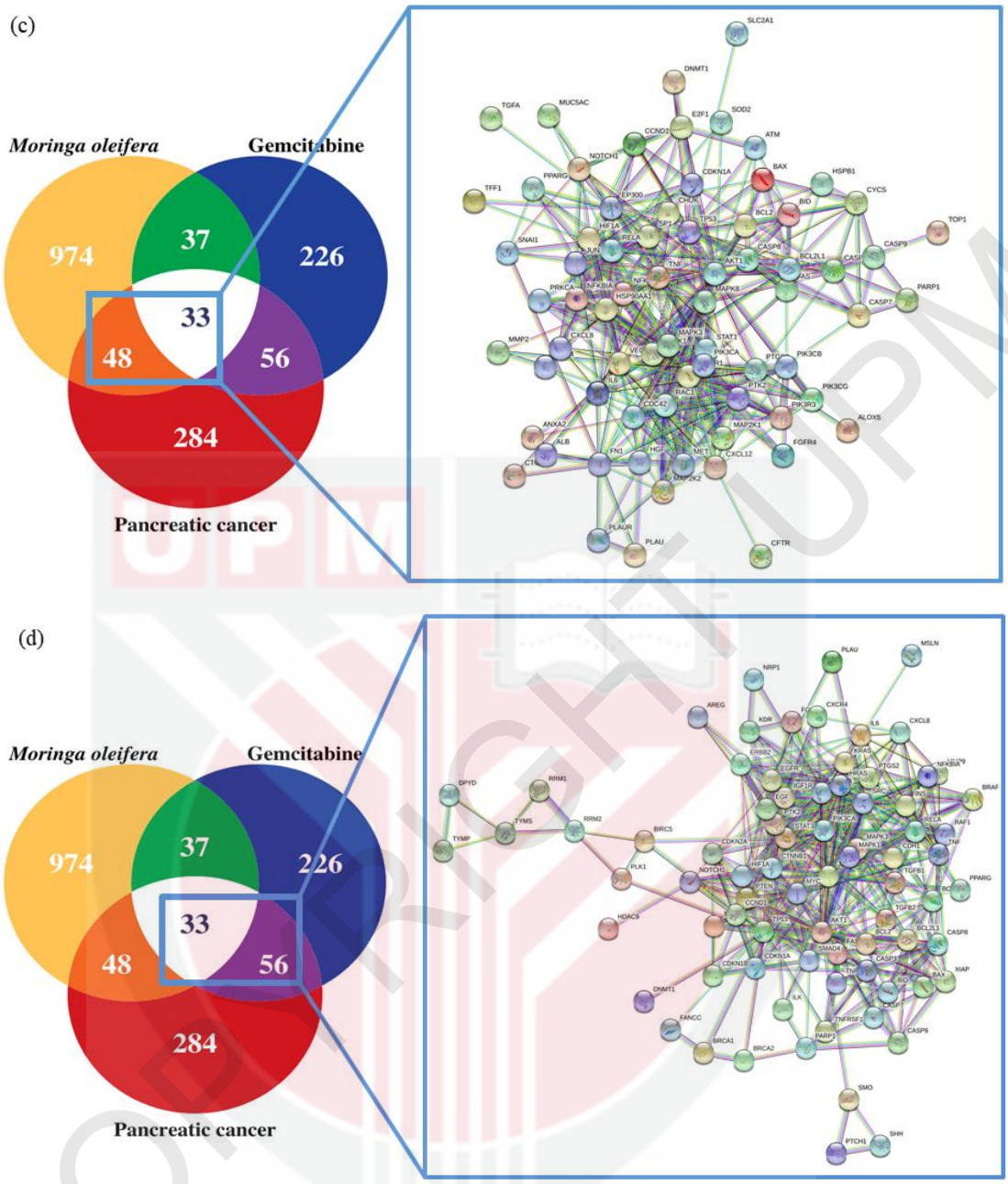

Figure 4.2. Targets of MO, GEM and their combinations against pancreatic cancer using STRING database. (a) Venn diagram and PPI network of MO+GEM-intersection target genes against pancreatic cancer. (b) Venn diagram and PPI network of shared biotargets-intersection against pancreatic cancer. (c) Venn diagram and PPI network of MO-intersection target genes against pancreatic cancer. (d) Venn diagram and PPI network of GEM-intersection target genes against pancreatic cancer.

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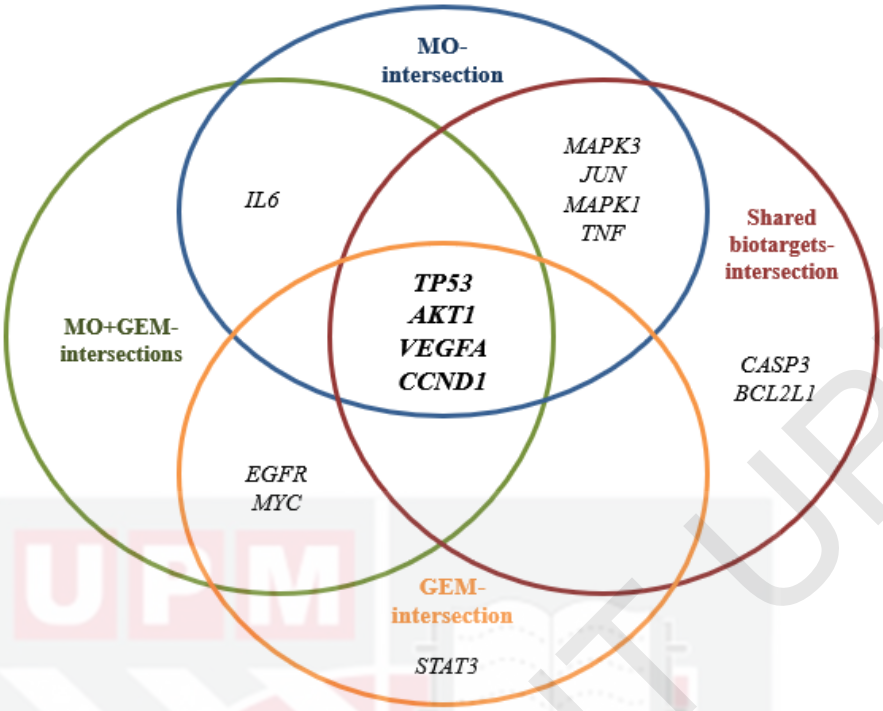

Figure 4.3. Hub genes derived from the PPI network based on degree, closeness and betweenness values. TP53, AKT1, VEGFA and CCND1 are the hub genes that overlapped between the 4 intersection networks against pancreatic cancer.

4.5 GO and pathway enrichment analysis

GO enrichment analysis was performed to further investigate the biological process, cellular component and molecular function of the pancreatic cancer-related target of MO and GEM using DAVID bioinformatics resources. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was also conducted to further reveal the pharmacological mechanism of the combination between MO and GEM against pancreatic cancer. The GO enrichment terms and KEGG pathway analysis are illustrated in (Figure 4.4 and 4.5).

The results of GO for MO+GEM-intersection against pancreatic cancer indicated that the top 10 biological process were mainly involved in negative regulation of apoptotic process, response to drug, positive regulation of cell proliferation, positive regulation of transcription, DNA-templated, positive regulation

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of gene expression, positive regulation of ERK1 and ERK2 cascade, response to estradiol, angiogenesis, positive regulation of transcription from RNA polymerase II promoter and positive regulation of protein phosphorylation. In cellular component enrichment analysis, target genes that were engaged in the top 10 terms include the cytosol, nucleus, cytoplasm, extracellular space, focal adhesion, nucleoplasm, protein complex, cell surface, plasma membrane and platelet alpha granule lumen. The top 10 molecular function enrichment was associated with the protein binding, identical protein binding, enzyme binding, kinase activity, transcription factor binding, protein heterodimerization activity, protein kinase binding, growth factor activity, protein tyrosinase kinase activity and protein phosphatase binding (Figure 4.4a). Refer Appendix XI for details of GO enrichment terms of MO+GEM-intersection.

The GO results showed that the top 10 biological process for shared biotargets-intersection were associated with negative regulation of apoptotic process, positive regulation of apoptotic process, response to drug, cellular response to DNA damage stimulus, extrinsic apoptotic signaling pathway in absence of ligand, activation of cysteine-type endopeptidase activity involved in apoptotic process, negative regulation of anoikis, regulation of cell proliferation, apoptotic process and release of cytochrome c from mitochondria. The top 10 significant enrichment terms represented by cellular component were involved in cytosol, nucleus, nucleoplasm, mitochondrion, death-inducing signaling complex, protein complex, mitochondrial outer membrane, cytoplasm, transcription factor complex and Bcl-2 family protein complex. For molecular function, the top 10 enrichment terms include identical protein binding, transcription factor binding, enzyme binding, protein binding, ubiquitin protein ligase binding, cysteine-type endopeptidase activity involved in apoptotic process, protein heterodimerization activity, protein kinase binding, BH3 domain binding and protein

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complex binding (Figure 4.4b). Refer Appendix XII for details of GO enrichment terms of shared biotargets-intersection.

The top 10 significant enrichment terms of biological process for MO-intersection targets against pancreatic cancer were involved in negative regulation of apoptotic process, response to drug, angiogenesis, intrinsic apoptotic signaling pathway in response to DNA damage, vascular endothelial growth factor receptor signaling pathway, platelet activation, positive regulation of transcription from RNA polymerase II promoter, Fc-epsilon receptor signaling pathway, positive regulation of neuron apoptotic process and positive regulation of peptidyl-serine phosphorylation. For cellular component, the top 10 enrichment terms include the cytosol, nucleus, phosphatidylinositol 3-kinase complex, focal adhesion, mitochondrion, nucleoplasm, cell-cell junction, cytoplasm, extracellular space and protein complex. The top 10 significant enrichment terms represented by molecular function were associated with identical protein binding, protein binding, enzyme binding, transcription factor binding, kinase activity, 1-phosphatidylinositol-3-kinase activity, protein kinase binding, histone deacetylase binding, protein heterodimerization activity and protein serine/threonine kinase activity (Figure 4.4c). Refer Appendix XIII for details of GO enrichment terms of MO-intersection.

For GEM-intersection targets against pancreatic cancer, the top 10 biological process were mainly engaged in negative regulation of apoptotic process, positive regulation of cell proliferation, response to drug, positive regulation of transcription, DNA-templated, response to estradiol, positive regulation of apoptotic process, positive regulation of gene expression, cell proliferation, positive regulation of MAP kinase activity and activation of cysteine-type endopeptidase activity involved in apoptotic process. The top 10 significant enrichment terms represented by cellular

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component were associated with cytosol, cytoplasm, nucleus, nucleoplasm, protein complex, cell surface, mitochondrion, caveola, focal adhesion and extracellular space. For molecular function, the top 10 enrichment terms include protein binding, identical protein binding, enzyme binding, protein heterodimerization activity, transcription factor binding, kinase activity, protein kinase activity, protein complex binding, protein phosphatase binding and protein homodimerization activity (Figure 4.4c). Refer Appendix XIV for details of GO enrichment terms of GEM-intersection.

In GO analysis comparison, the top 10 biological process among the 4 intersection networks showed that the negative regulation of apoptotic process was highly regulated compared to the other processes. In cellular component enrichment analysis, the top 10 significant enrichment terms were mainly involved in the cytosol among the 4 intersection networks. The top 10 significant enrichment terms represented by molecular function showed that protein binding was highly regulated in MO+GEM- and GEM-intersections while identical protein binding was highly regulated in shared biotargets- and MO-intersections (Figure 4.4).

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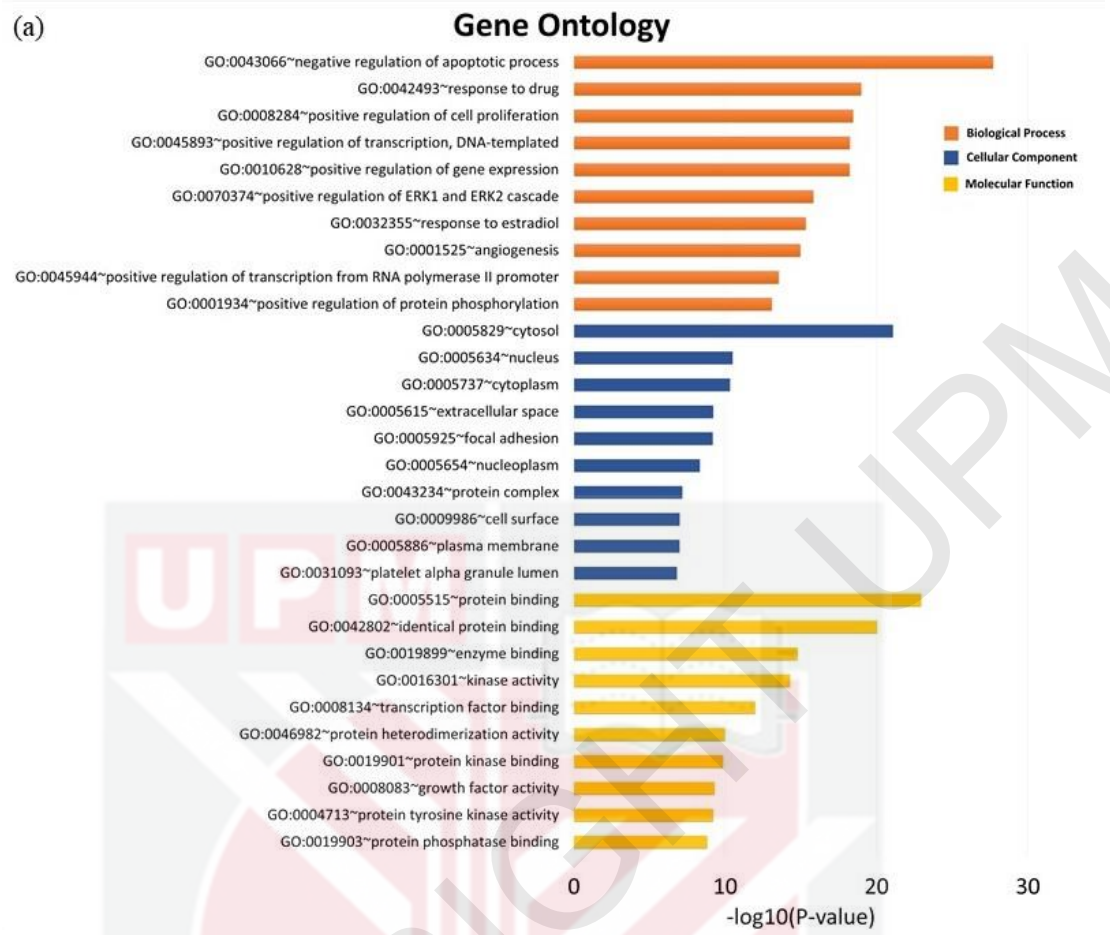

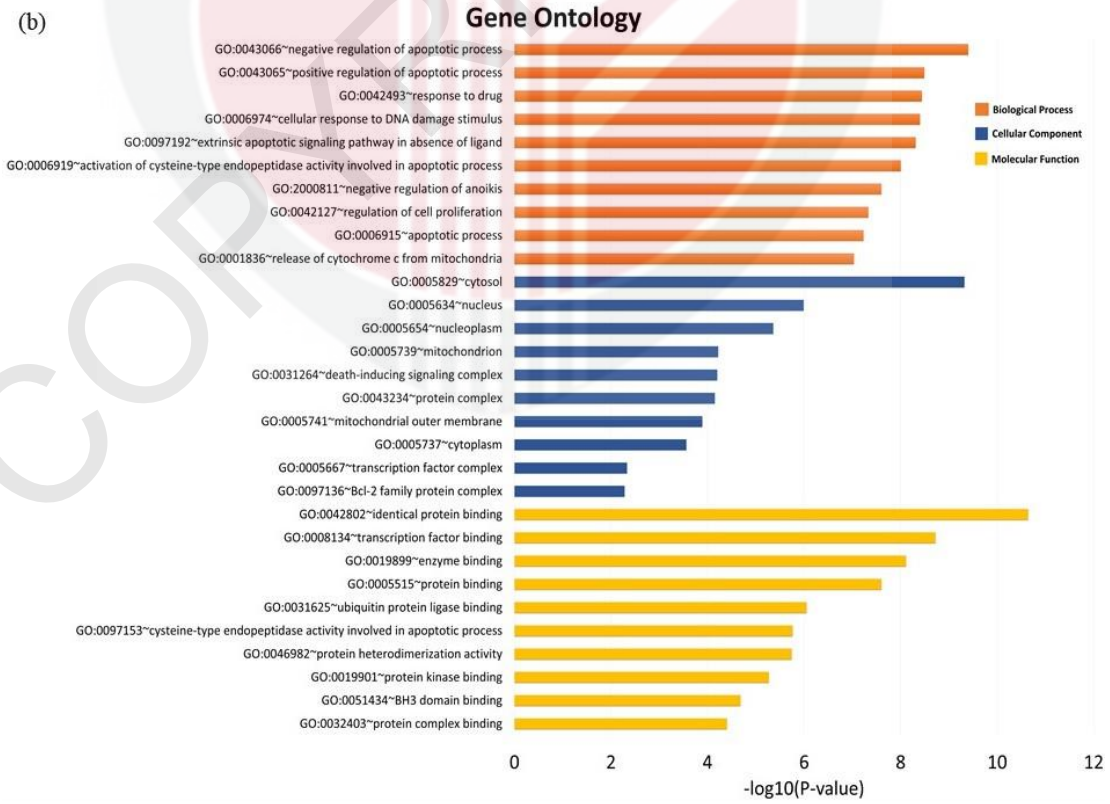

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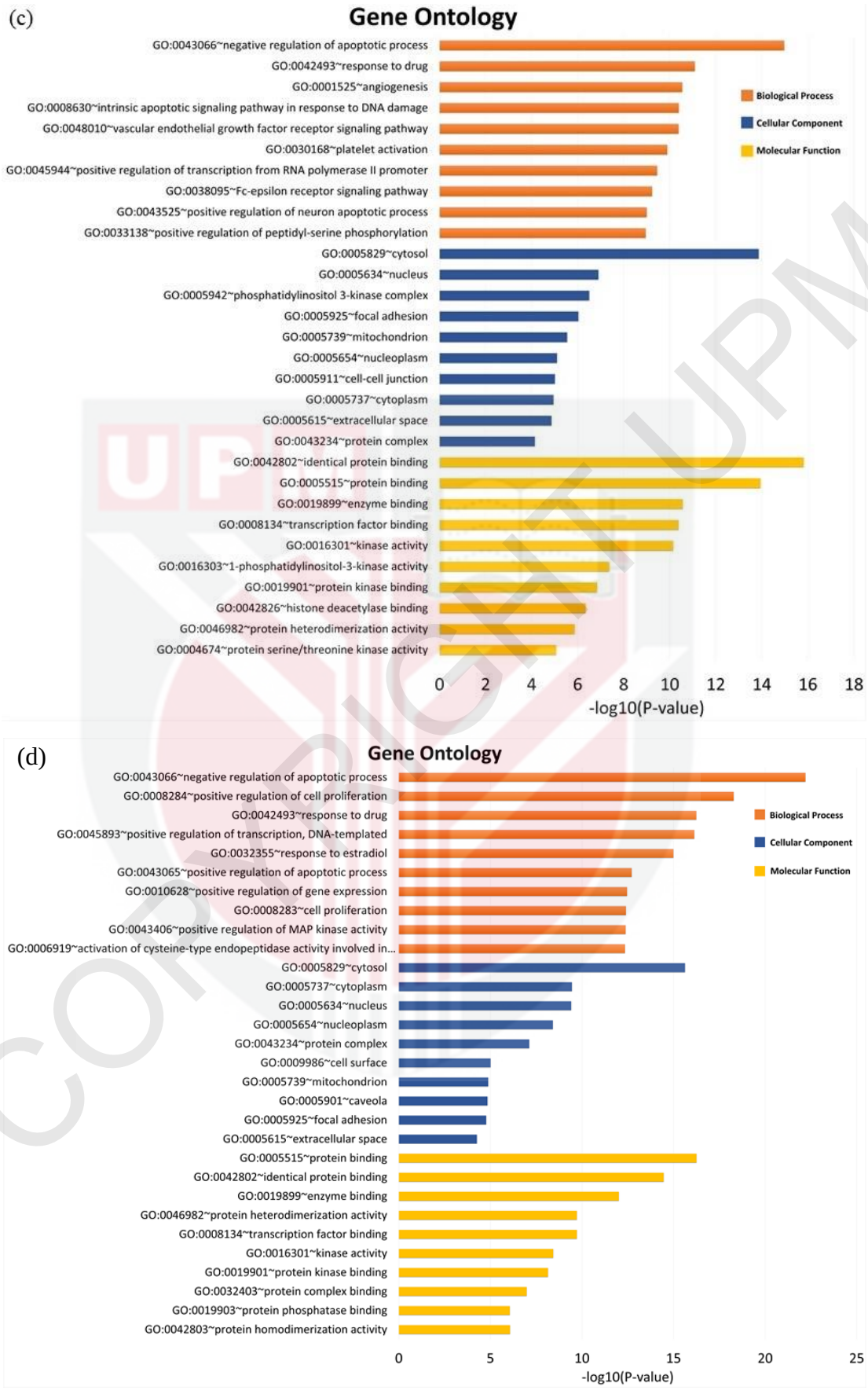

Figure 4.4. GO enrichment terms analysis of MO, GEM and their combinations against pancreatic cancer. (a) The bar chart represents the top 10 significant enrichment

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terms represented by the biological process, cellular component and molecular function of MO+GEM-intersection target genes against pancreatic cancer. (b) The bar chart represents the top 10 significant enrichment terms represented by the biological process, cellular component and molecular function of shared biotargets-intersection against pancreatic cancer. (c) The bar chart represents the top 10 significant enrichment terms represented by the biological process, cellular component and molecular function of MO-intersection target genes against pancreatic cancer. (d) The bar chart represents the top 10 significant enrichment terms represented by the biological process, cellular component and molecular function of GEM-intersection target genes against pancreatic cancer.

Besides, the results of top 10 KEGG pathways for MO+GEM-intersection against pancreatic cancer were involved in pathways in cancer, hepatitis B, pancreatic cancer, proteoglycans in cancer, prostate cancer, colorectal cancer, chronic myeloid leukemia, FoxO signaling pathway, bladder cancer and endometrial cancer (Figure 4.5a). In shared biotargets-intersection against pancreatic cancer, the results of top 11 KEGG pathways were engaged in pathways in cancer, hepatitis B, apoptosis, TNF signaling pathway, colorectal cancer, chagas disease (American trypanosomiasis), non-alcoholic fatty liver disease (NAFLD), proteoglycans in cancer, small cell lung cancer, prostate cancer and pancreatic cancer (Figure 4.5b). The top 10 KEGG pathway in the MO-intersection targets against pancreatic cancer were mainly involved in pathways in cancer, hepatitis B, apoptosis, pancreatic cancer, prostate cancer, proteoglycans in cancer, renal cell carcinoma, colorectal cancer, toll-like receptor signaling pathway and TNF signaling pathway (Figure 4.5c). For GEM-intersection target against pancreatic cancer, the top 10 KEGG pathways include pathways in cancer, hepatitis B, proteoglycans in cancer, bladder cancer, pancreatic cancer, prostate cancer, colorectal cancer, endometrial cancer, chronic myeloid leukemia and FoxO signaling pathway (Figure 4.5d). Refer Appendix XV for details of KEGG pathway enrichment analysis.

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In comparison, the KEGG pathway enrichment analysis of the 4 intersection networks were mainly associated with pathways in cancer and hepatitis B. Among the four networks, pancreatic cancer is one of the related pathways where it is ranked as third, fourth and fifth in the MO+GEM-intersection, MO-intersection and GEM-intersection, respectively. However, pancreatic cancer was found in the last rank of the shared biotargets-intersection (Figure 4.5).

The details of pancreatic cancer signaling pathway was shown in (Figure 4.6). The activating point mutations in the *KRAS* and overexpression of *HER-2/neu* occur early, while inactivation of the *p16* at an intermediate stage, and the inactivation of *p53*, *SMAD4*, and *BRCA2* occur relatively late. Mutations in *KRAS* oncogenes activate the PI3K-Akt signaling pathway producing the PIP3 protein. Subsequently, this pathway modulates downstream signaling cascades which include Rac-GEF and PKB/Akt signaling cascades. The activation of Rac by Rac-GEF signaling cascade cause the cytoskeleton remodeling. Besides, the activation of Rac and the phosphorylation of IKK by the activation of AKT promote the release of NF-κB which regulate the anti-apoptotic genes. The activation of AKT also inhibits the phosphorylation of BAD and CASP9 which lead to the survival of cell and apoptosis suppression, respectively. Furthermore, the activation of Raf in MAPK signaling pathway phosphorylate MEK, ERK and JNK. The activation of Jak*1* by EGFR and HER2/neu complex promotes the phosphorylation of STAT3 and STAT1 in Jak-STAT signaling pathway and subsequently activates the VEGF in VEGF signaling pathway stimulating angiogenesis. Other than that, *p16* tumor suppressor gene play a crucial role in the regulation of cell cycle. Mutations in this gene suppress the inhibition of CDK4/6-cyclin D1 complex. This action phosphorylates the Rb protein resulting in the dissociation of Rb-E2F complexes which then affect the normal DNA action and

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lead to G1/S progression. DNA damage may lead to *p53* mutation, which will affect the regulation of expression of the six genes (*p21*, *Bax*, *p48*, *GADD45*, *Bak* and *POLK*) and lead to the uncontrolled proliferation, increased survival of cancer cells as well as genomic instability. The ligand binding of TGFβ to the TGFβR1 and TGFβR2 activates the phosphorylation of SMAD2/3 which forms complexes with SMAD4 which serve as a key mediator in TGFβ signaling pathway. However, the inactivation of *SMAD4* tumor suppressor gene leads to loss of growth inhibitory effect of TGFβ. B*RCA2* mutations impair the DNA damage response and homologous recombination by disrupting the Rad51-BRCA2 interaction. This action inhibits the double-strand break repair which leads to failure in repairing the genes.

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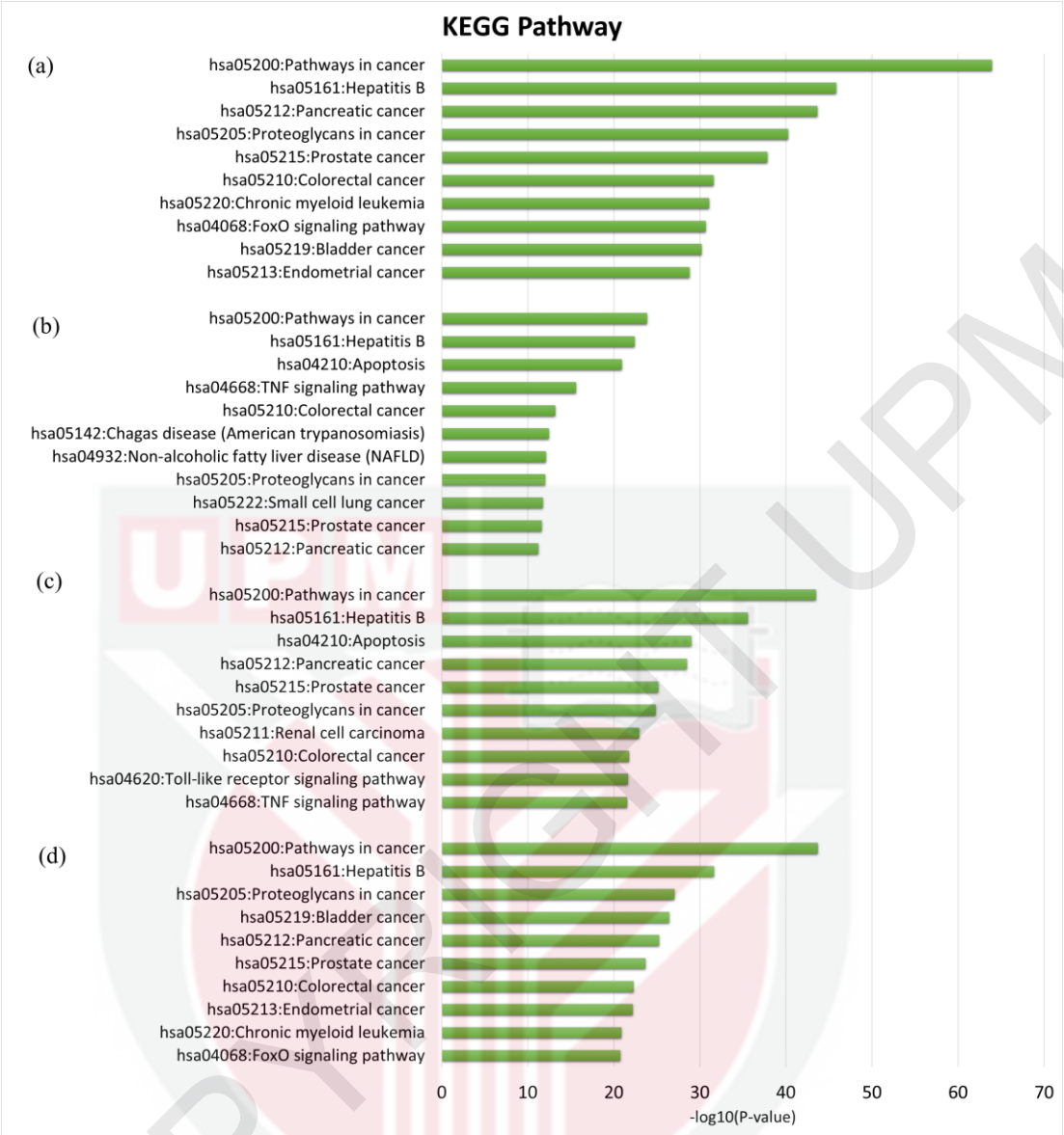

Figure 4.5. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of MO, GEM and their combinations against pancreatic cancer. (a) The bar chart represents the top 10 KEGG pathway analysis of MO+GEM-intersection target genes against pancreatic cancer. (b) The bar chart represents the top 11 KEGG pathway analysis of shared biotargets-intersection against pancreatic cancer. (c) The bar chart represents the top 10 KEGG pathway analysis of MO-intersection target genes against pancreatic cancer. (d) The bar chart represents the top 10 KEGG pathway analysis of GEM-intersection target genes against pancreatic cancer.

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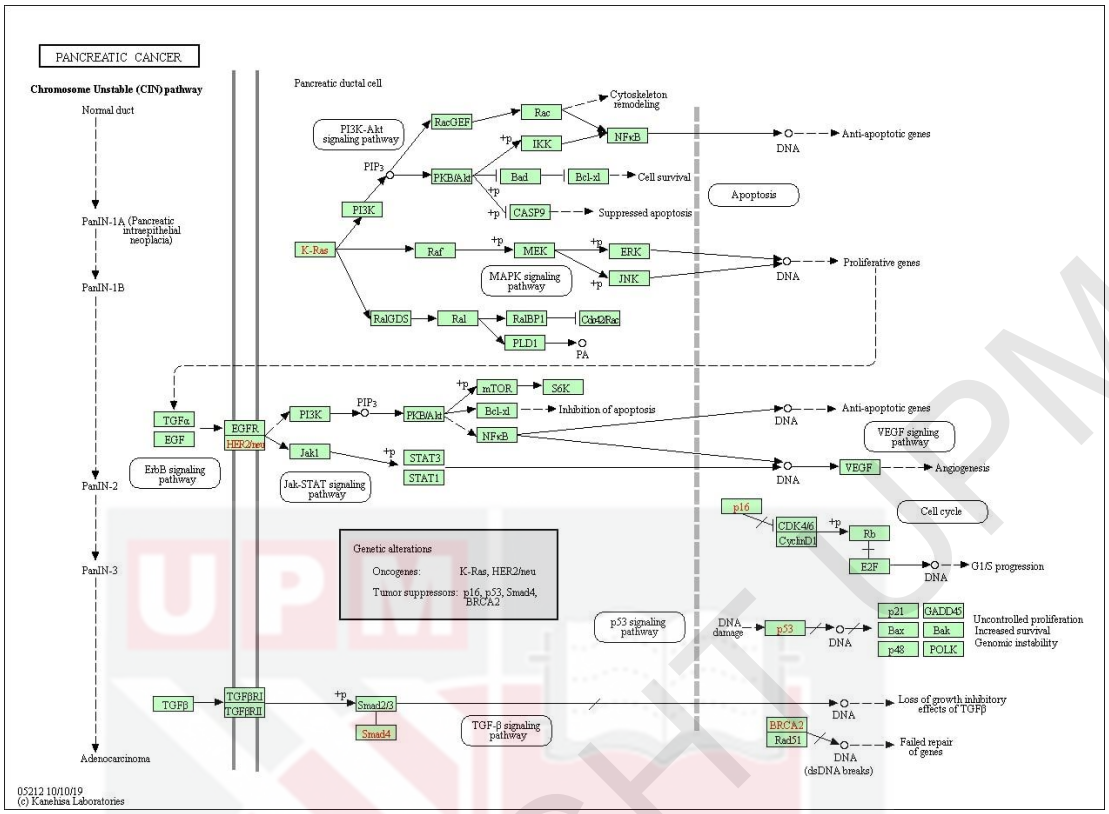

Figure 4.6. Diagram of the pancreatic cancer pathway targeted by the combination of MO and GEM from KEGG pathway map. The genes in red text are the target genes of MO and GEM in pancreatic cancer including the *KRAS* and *HER2/neu* as the oncogenes and *p16, p53, SMAD4* and *BRCA2* as the tumor suppressors genes. The activation of *KRAS* and *HER2/neu* involve in multiple effector pathways such as PI3K-AKT signaling pathway, MAPK signaling pathway, ErbB signaling pathway, JAK-STAT signaling pathway and VEGF signaling pathway. Inactivation of *SMAD4* causes the loss of inhibitory influence of TGF-β signaling pathway. The inactivation of *p16* will affect the normal cell cycle. Mutations in *p53* and *BRCA2* likely contribute to extensive genomic instability and aneuploidy.

4.6 Compound-target-pathway network construction

The compound-target-pathway network (Figure 4.7) was constructed based on the significantly enriched pathways by using Cytoscape software to further understand the pharmacological mechanism of MO and GEM against pancreatic cancer. Compound-target network analysis identified catechin, kaempferol and quercetin as hub compounds in the network of MO+GEM-intersection target genes against pancreatic cancer since the degree value of these compounds is more than average

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degree of node, 14.910. In the network of shared biotargets-intersection against pancreatic cancer with the average degree of node, 22.737, only catechin was recognized as hub compound while two hub compounds which are catechin and kaempferol were identified in the network of MO-intersection target genes against pancreatic cancer with degree value more than average degree of node, 30.909 (Table 4.3).

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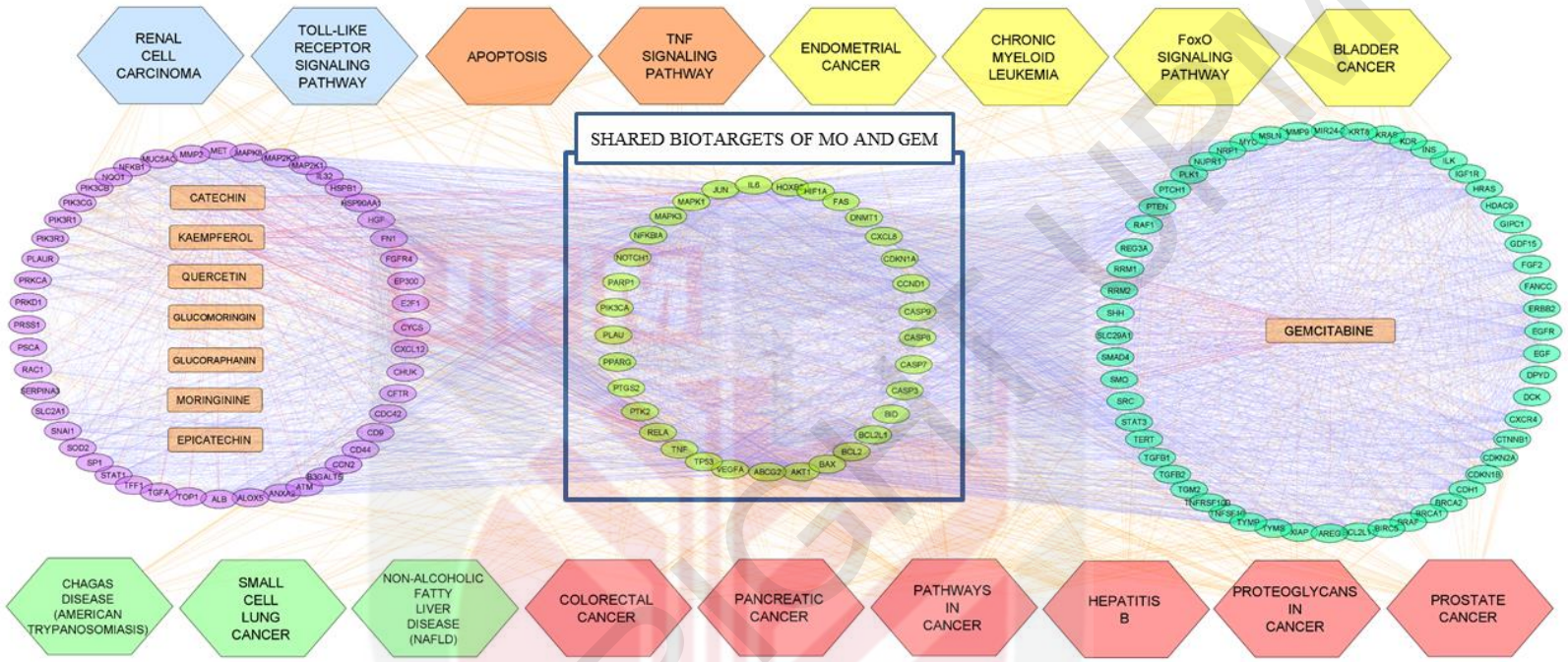

Figure 4.7. The compound-target-pathway network of the MO, GEM and their combinations against pancreatic cancer by using Cytoscape. The network contains 162 nodes (8 compounds, 17 pathways and 137 target genes) and 3227 edges. The left and right compound-target network are the MO- and GEM-intersections target genes, respectively while the center compound-target network is their combinations against pancreatic cancer. The light blue hexagons represent the pathways involved in MO-intersection targets, the orange hexagons represent the pathways involved in the MO- and shared biotargets-intersections, the yellow hexagons represent the pathways involved in the GEM-intersection targets, the green hexagons represent the pathway involved in shared biotargets-intersection and the pink hexagons represent the pathway involved in three intersection networks. The oval nodes represent the target genes, the rectangle nodes represent the compounds, the hexagon nodes represent the signaling pathway while the edges represent the interaction between them.

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Table 4.3. Hub compounds in compound-target network based on the degree values.

	Compounds	Degree
MO+GEM-intersection target genes against pancreatic cancer	Gemcitabine	89
	Catechin	46
	Kaempferol	43
	Quercetin	18
Shared biotargets-intersection against pancreatic cancer	Gemcitabine	33
	Catechin	27
MO-intersection target genes against pancreatic cancer	Catechin	47
	Kaempferol	42

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CHAPTER 5

DISCUSSION

As the 7th most common cause of cancer death, most pancreatic cancer patients are less likely to survive for more than 5 years since the relative survival rate at 5 years for pancreatic cancer is less than 10% across the globe. Traditionally, conventional approach in drug discovery is considered as "one drug, one target, one disease" theory. In contrast, network pharmacology study has been known as "multi-drug, multi-target, multi-diseases" to explore the correlation of drugs and diseases (Sakle et al., 2020). In this present study, network pharmacology approach was proposed to identify the potential bioactive compounds and theoretically evaluate the effect of the combination therapy of MO and GEM against pancreatic cancer.

The progression of pancreatic cancer is often associated with the mutation of oncogene and tumor suppressor genes such as *KRAS* and *TP53*, respectively. Currently, there is no effective treatment that has been clinically approved which directly targets the mutation of *KRAS* oncogene in pancreatic cancer (Ryu et al., 2021). Despite the significant efforts in the research and development of the drug, gemcitabine (GEM) which is the gold standard first-line chemotherapy against locally advanced and metastatic pancreatic cancer since 1997 has poor efficacy due to the rapid acquired resistance (Kang et al., 2018). It has been reported in a previous study that pancreatic cancer patients with *KRAS* mutation had a poor response and shorter survival rates compared to those with wild-type *KRAS* after being treated with gemcitabine (Ryu et al., 2021). Besides, mutant *TP53* has been demonstrated to induce gemcitabine resistance towards pancreatic cancer cells. Gemcitabine stabilized the mutant of p53 protein in the nucleus and caused

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chemoresistance which occurred concurrently with the mutant p53-dependent expression of *CDK1* and *CCNB1* genes that led to the hyperproliferation of pancreatic cancer cells (Fiorini et al., 2015). Hence, this present *in silico* study combined GEM with MO that might help the future researchers in developing an effective treatment against pancreatic cancer.

This study revealed 4 potential bioactive compounds in MO including catechin, epicatechin, kaempferol and quercetin that met the requirement of ADME screening. Glucomoringin, glucoraphanin and moringinine were included in this study as they have been found to exhibit the anticancer effect in various kind of cancer (Ali et al., 2016; Almuhayawi et al., 2020; Rajan et al., 2016). However, only 3 compounds of MO which are catechin, kaempferol and quercetin that were considered as the hub compounds in MO+GEM-intersection target genes against pancreatic cancer. Catechin and kaempferol were also identified to be the hub compounds in MO-intersection targets against pancreatic cancer. Kaempferol was found to play a role in the regulation of various cancer cell activities which include inflammation, apoptosis and cell cycle (Lee & Kim, 2016). Quercetin had been reported to stimulate anti-tumor activities through the induction of apoptosis, cell cycle and autophagy (Mouria et al., 2002; Psahoulia et al., 2007; Senthilkumar et al., 2011; Vidya Priyadarsini et al., 2010). Since catechin is the only compound that has been recognized as a hub compound in the shared biotargets-intersection against pancreatic cancer, this compound plays a vital role against pancreatic cancer. Catechin is one of the flavonoid derivatives which is prominently found in green tea that possess health-promoting properties including anticancer and antioxidant (Bae et al., 2020; Musial et al., 2020; Haiyan Sun et al., 2020). It had been demonstrated that

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catechin which is also known as cyclooxygenase-1 (COX-1) inhibitor had exhibited a synergistic and additive anti-proliferative effect on breast, bladder and prostate cancer *in vitro* with the combination of COX-2 inhibitor (Farivar-Mohseni et al., 2004; McFadden et al., 2006). Besides, catechin with the combination of IP6 also give a reduction in cellular proliferation as well as induced the apoptosis effect in the pancreatic cancer cells, MiaPaca and Panc-1, significantly (McMillan et al., 2007).

Several hub genes were identified based on the PPI network and considered to play a crucial role within the network against pancreatic cancer (Wu et al., 2020). In this study, it is suggested that *TP53, AKT1, VEGFA* and *CCND1* may be the key targets for anti-cancer activity of MO and GEM combination, which might major biological importance in treating pancreatic cancer. *TP53* has been known to be mutated in 70% of cases in pancreatic cancer causing the loss of DNA binding ability which activates the transcription of genes (Cicenas et al., 2017). The progression of malignant pancreatic cancer was found to be associated with the sustained expression of mutant *TP53* (Jahedi et al., 2019). From the previous study, R273H mutation of P53 in MiaPaca-2 pancreatic cancer cell lines has been found to play roles in increasing colony formation and proliferation and resistance to drug (Jahedi et al., 2019). Study on MO demonstrated that the hot water leaf extract of the plant revealed the antiproliferative effect in the lung cancer cell, A549, by the activation of P53, caspases and PARP1 cleavage which led to the apoptosis of the cancer cell (Tiloke et al., 2018). Besides, *TP53* plays a role as a diagnostic marker in pancreatic cancer patients treated with GEM chemotherapy (Sinn et al., 2020). Previously, study has reported that the chemoresistance of GEM can be overcomed by the combination of P53-reactivating molecules, RITA and CP-31398, which reduced the growth rate and caused

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apoptosis to the pancreatic cancer line (Fiorini et al., 2015). Furthermore, *AKT1* is the main gene in the signaling pathway of PI3K/Akt and in the various types of cancer. The abundance of *AKT1* activity has been shown through transmission of strong anti-apoptosis signals (Parsons et al., 2010). It has been demonstrated that activation of AKT protein was prevented by the AKT inhibitor together with GEM which then enhanced the cytotoxic activity of GEM (Wang et al., 2020). In pancreatic cancer, *VEGFA* is the most common and prevalent angiogenic factor among other family members. Since it can promote angiogenesis and greatly increases the cancer cell motility, *VEGFA* is responsible for inducing pancreatic cancer cell invasion and migration (Costache et al., 2015; Doi et al., 2012). The suppression of *VEGFA* expression by GEM will cause the inhibition of tumor angiogenesis in the cancer cells (Ikeda et al., 2010). Researchers recently are also focusing to target the pancreatic stroma as the tumor microenvironment of pancreatic cancer play an important role in its pathogenesis. Previous study has reported an aberrant gene expression profile in the cancer-associated stroma, which is associated with high expression of VEGFA, COX-2 and collagen I as well as the alteration of integrin expression. These could result in the abnormal interaction of epithelial-mesenchymal that enhance the proliferation and invasiveness of cancer cells (Korc et al., 2007). Hence, it is suggested that the MO and GEM combination may help in the inhibition of aberrant signal generated by pancreatic cancer associated stromal cells and provides therapeutic options. Cyclin D1, encoded by *CCND1* gene is a key regulator protein in the G1-S phase transformation which is also responsible for the regulation of cell growth and differentiation. Cyclin D1 deregulation can result in tumor formation caused by genetic instability *in vivo* and *in vitro* (Bachmann et al., 2015). Study has shown that GEM causes the *CCND1* suppression in the human breast cancer cell line which is due to the cell cycle

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arrest in S phase (Hernández-Vargas et al., 2007). Moreover, cancer cells that have been treated with the leaf extract of MO were shown to have a significant reduction of cyclin D1 levels in a dose-dependent manner (Kou et al., 2018). Therefore, it is assumed that downregulation of mutant *TP53, AKT1, VEGFA* and *CCND1* genes might reduce the ability of pancreatic cancer cells to survive.

Besides, this study also demonstrated that the combination of MO and GEM might have a greater effect in targeting the pancreatic cancer genes rather than MO or GEM alone. This can be seen where the combination of MO and GEM can target other important functional genes that are not present in GEM alone or MO alone including *CASP3* and *BCL2L1*. Previous study has shown that apoptosis of BxPC-3 pancreatic cancer cells induced by gemcitabine is caused by the *CASP3* stimulation, PARP cleavage and the increased number of cells in the sub-G0 (Chandler et al., 2004). In contrast, BCL-xL which is encoded by the *BCL2L1* gene is an important anti-apoptotic protein that aids in the survival of pancreatic cancer cell differentiation (Loo et al., 2020). Hence, these genes could be an ideal target in the combination between MO and GEM against pancreatic cancer.

Furthermore, the possible mechanism of action and signaling pathway against pancreatic cancer was further evaluated through enrichment analysis. The result of the GO enrichment analysis demonstrated that the most enriched biological process of the target genes of MO as well as its combination with GEM involves in the regulation of cell proliferation and apoptosis. It has been reported that the positive and negative regulation of apoptotic processes are simultaneously activated through noxious and protective signal via various pathways (Solary et al., 2000). Thus, it is pertinent to finely balance the

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regulation of the two signals to increase the effectiveness of the combination therapy between MO and GEM. Besides, the positive regulation of cell proliferation might lead to the abnormal growth of the cancer cell. Therefore, by targeting the genes involved in the regulation of cell proliferation might improve the treatment of pancreatic cancer.

From the KEGG pathway enrichment analysis of MO as well as its combination with GEM, the results showed that the enrichment pathways were mainly related to pancreatic cancer, prostate cancer, colorectal cancer, bladder cancer, endometrial cancer and chronic myeloid leukemia. There was also involvement of other pathways in this study such as hepatitis B, apoptosis, FoxO signaling pathway, Ras signaling pathway, VEGF signaling pathway and toll-like receptor signaling pathway. On the other hand, when MO and GEM were combined, other diseases were also being targeted such as chagas disease (American trypanosomiasis) and non-alcoholic fatty liver disease (NAFLD). These findings revealed that "multi-pathway" theory has been applied. In pancreatic cancer pathway, other than *p53, p16, KRAS* and *SMAD4* genes that were commonly mutated, the mutation of *BRCA2* gene is also involved in this pathway. Findings exhibited that genetic alterations may be the cause of the occurrence in 10% of pancreatic cancer cases from inherited syndrome which include the *BRCA2* gene mutation (Tersmette et al., 2001). This mutation impairs the homologous recombination and response of DNA damage by direct *RAD51* regulation (Martinez-Useros & Garcia-Foncillas, 2016). Furthermore, the mutation of *BRCA2* is also involved in the changes of DNA repair which may act as DNA damage repair deficiency and genomic instability biomarkers (Martinez-Useros & Garcia-Foncillas, 2016).

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CHAPTER 6

CONCLUSION, LIMITATION AND RECOMMENDATION

In conclusion, this is the first network pharmacology study reported to predict the pancreatic cancer target genes of MO bioactive compounds in the combination with GEM and theoretically evaluate their effect in pancreatic cancer development and progression. Catechin was the main hub compound found in MO as well as its combination with gemcitabine that stimulates multiple targets including TP53, AKT1, VEGFA and CCND1 in targeting pancreatic cancer. Furthermore, these genes were found to be functionally enhanced in multiple pathways associated with pancreatic cancer development. Also, CASP3 and BCL2L1 that are not targeted by MO or GEM, were found in the shared targets of MO and GEM, which may represent the new target protein against pancreatic cancer. Hence, the effect of GEM could be enhanced when it is combined with MO as they can target genes that are not targeted by MO or GEM alone against pancreatic cancer. The approaches that were applied in this study have provided knowledges on discovering the multi-compounds, multi-targets and multi-pathways as well as new insights into the anti-pancreatic cancer effects of the MO and GEM combination therapy. However, this *in-silico* study only provides a predictive overview of the combination therapy in treating pancreatic cancer. Therefore, further *in vitro* and *in vivo* experimental validations and subsequent clinical applications are required as the recommendations to verify these findings in the future.

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APPENDICES I

11.1 *In silico* screening of bioactive compound of MO

- Go to TCMSP database https://www.tcmspw.com/tcmsp.php, choose chemical name and insert the name of the chemical: Catechin

- Choose the compound interest from the list given

- The value for oral bioavailability (OB), drug- likeness (DL) and Caco-2 permeability shown in the red boxes

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- Compound that met the requirement of OB ≥ 30%, Caco-2 permeability ≥ −0.4 and DL ≥ 0.18 were considered as the bioactive compound of *Moringa oleifera*

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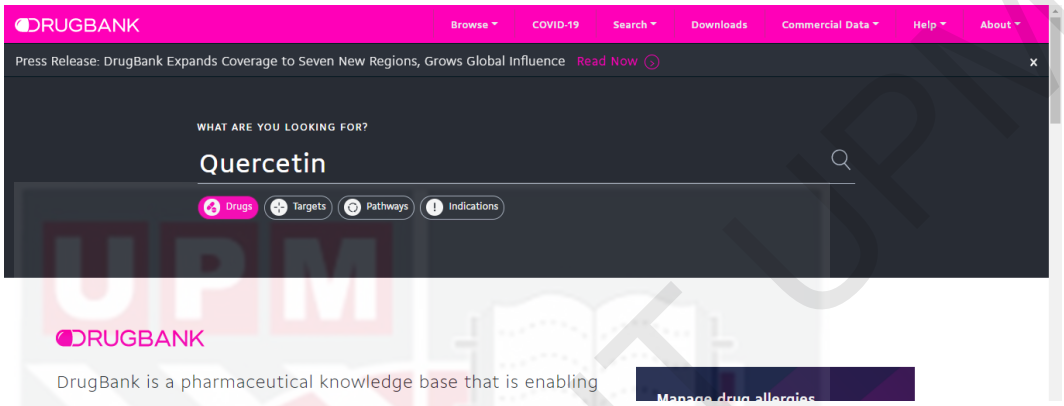
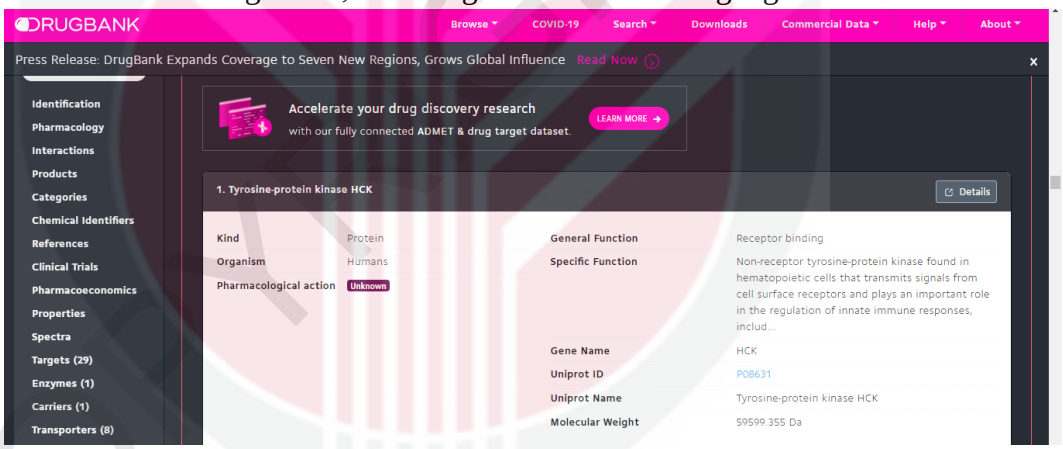
APPENDICES II

11.1 Target genes of the bioactive compound in MO

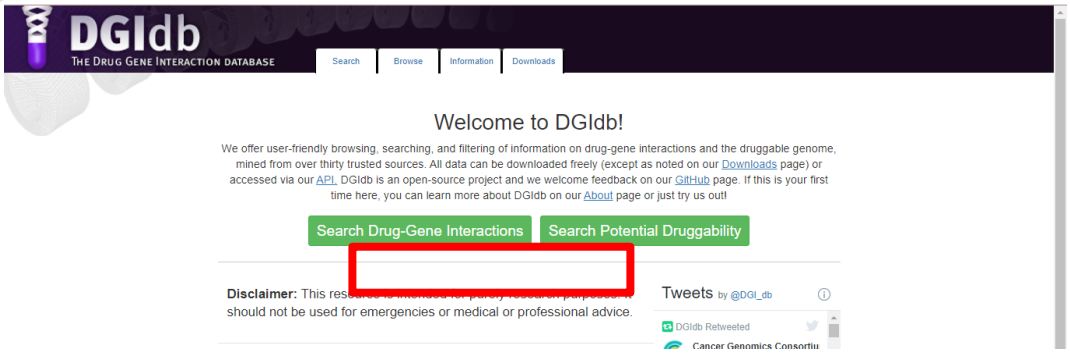
- **Drugbank:** https://go.drugbank.com/
  - Insert the name of compound



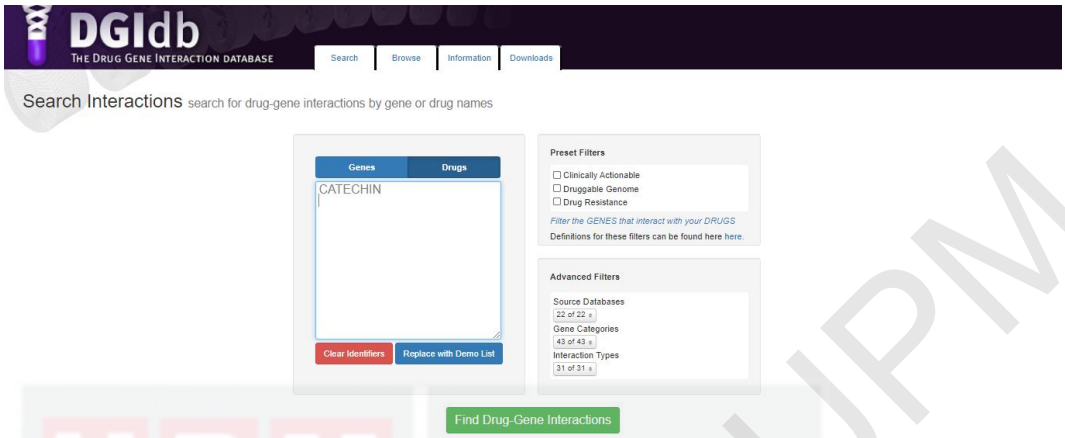
  - From the right box, click targets. The related target genes will be shown.



- **The Drug Gene interaction Database (DGIdb):** https://www.dgidb.org/
  - Click search drug-gene interactions

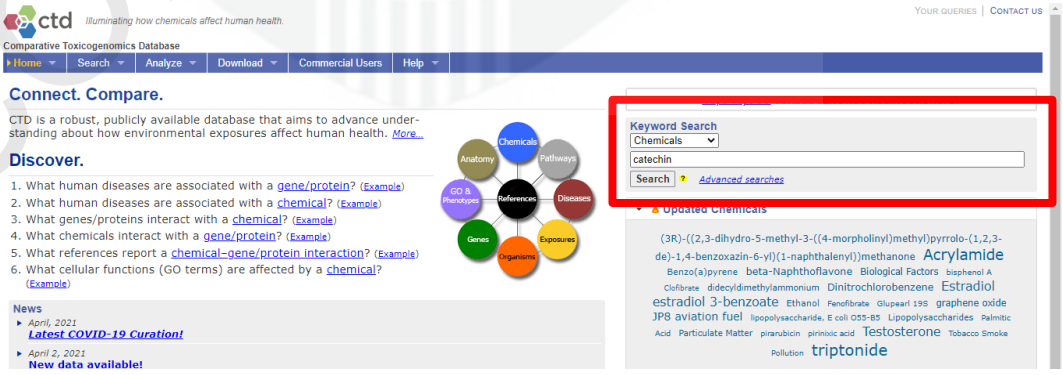


- Click drug, enter the compound name and click find drug-gene interaction



- The target genes will be shown

- **Comparative Toxicogenomics Database (CTD):** http://ctdbase.org/
  - Change the keyword search to chemical, enter the compound name and click search



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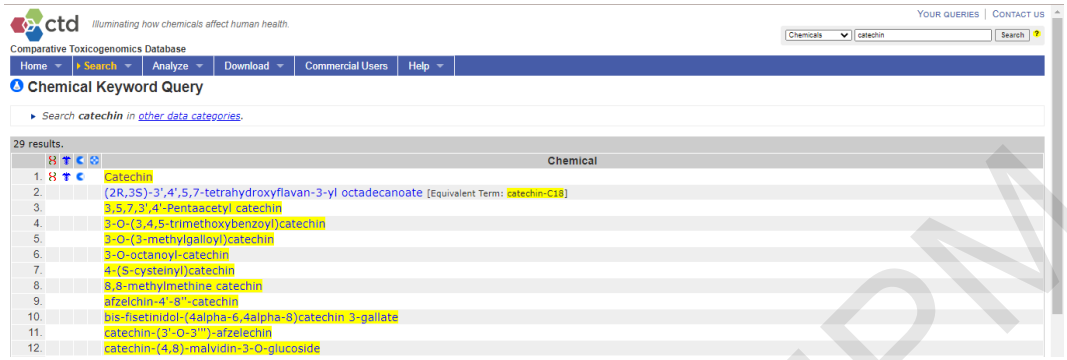
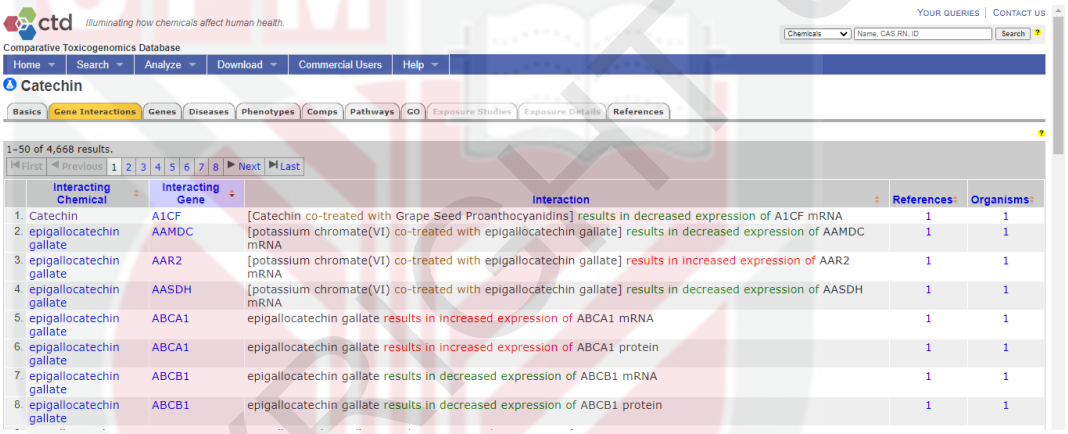
- The search results will be shown. Click on the compound interest



- Click gene interaction to see the related genes and it can be downloaded at the bottom of the page



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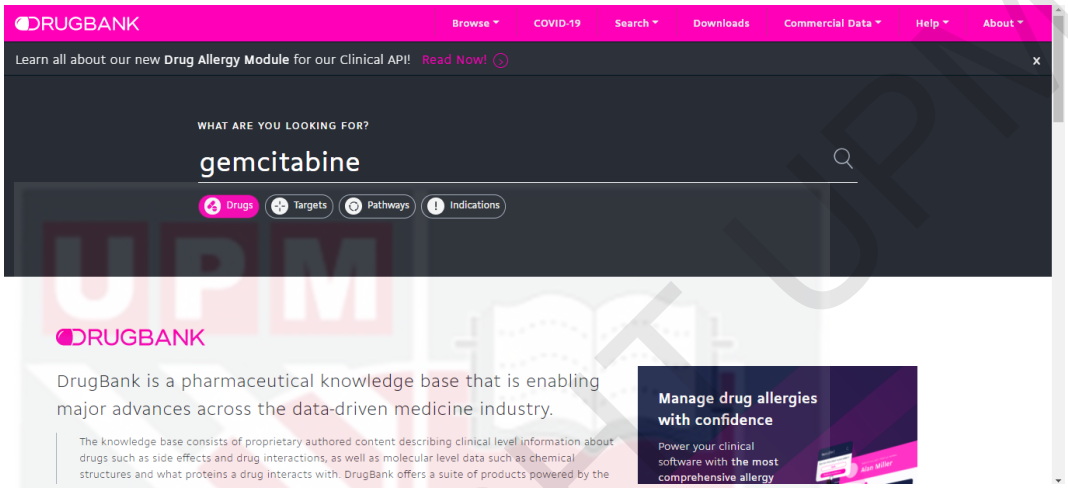
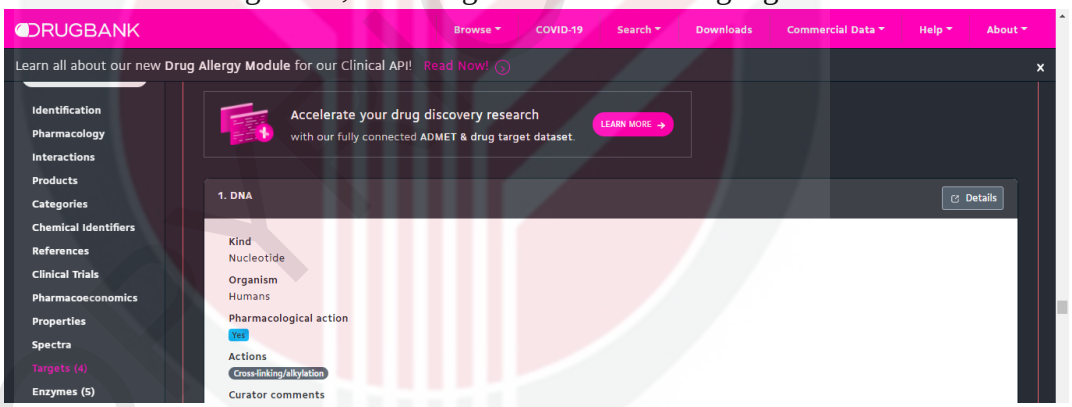
APPENDICES III

11.1 Target genes of GEM

- **DrugBank:** https://go.drugbank.com/
  - Enter the drug name



  - From the right box, click targets. The related target genes will be shown.



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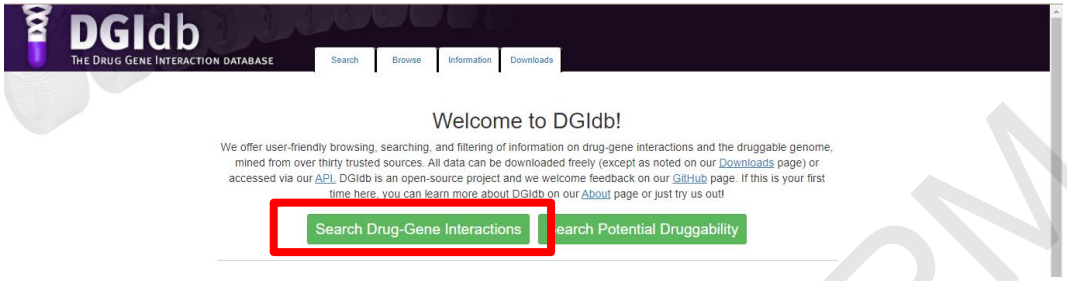
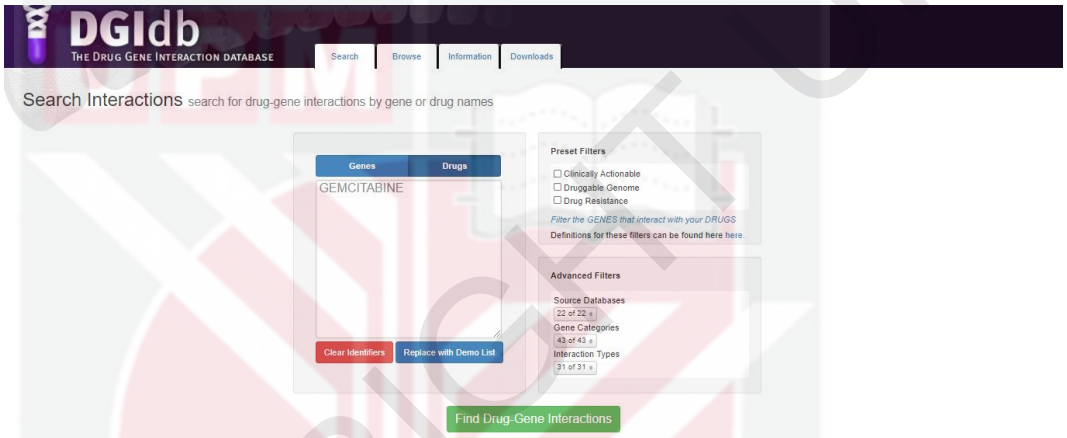
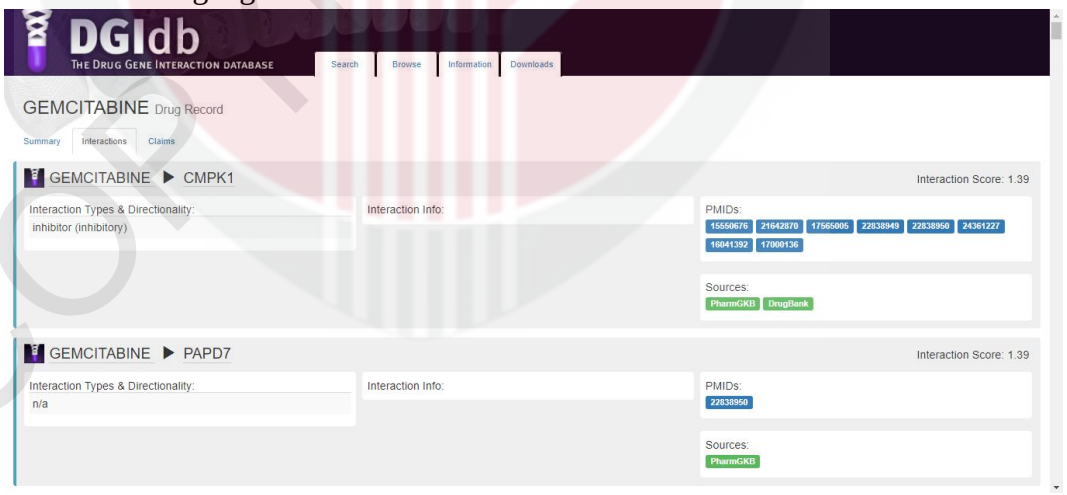
- **The Drug Gene interaction Database (DGIdb):** https://www.dgidb.org/
  - Click search drug-gene interactions



  - Click drug, enter the compound name and click find drug-gene interaction



  - The target genes will be shown



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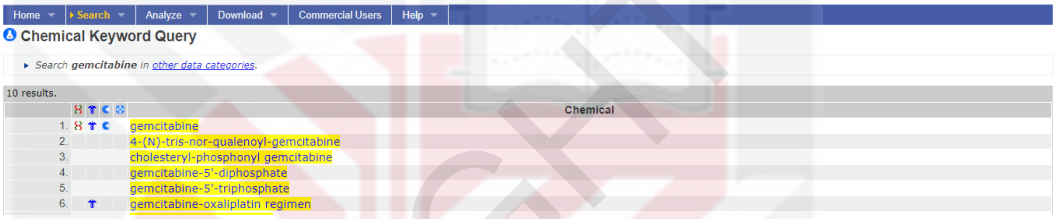
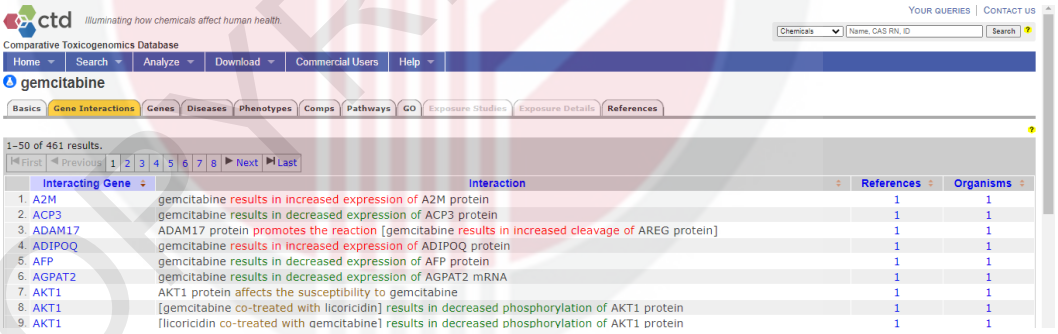
- **Comparative Toxicogenomics Database (CTD):** http://ctdbase.org/
    - Change the keyword search to chemical, enter the compound name and click search

    - The search results will be shown. Click on the compound interest



    - Click gene interaction to see the related genes and it can be downloaded at the bottom of the page



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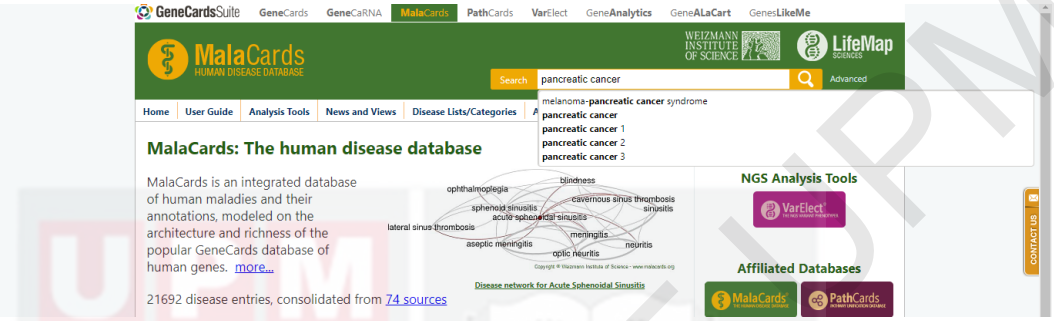
APPENDICE IV

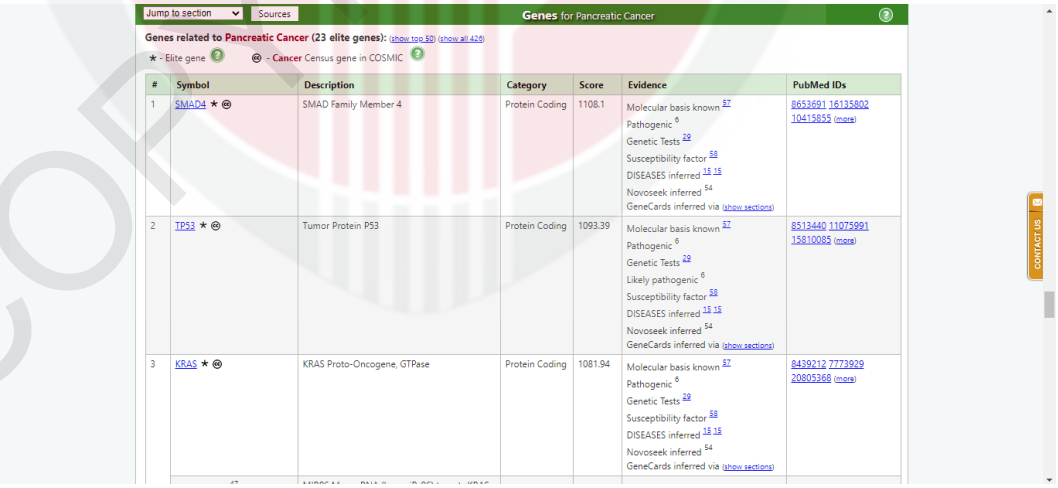
11.1 Target genes of pancreatic cancer

- **MalaCards database:** https://www.malacards.org/
  - Enter the disease name (Pancreatic cancer) and click search

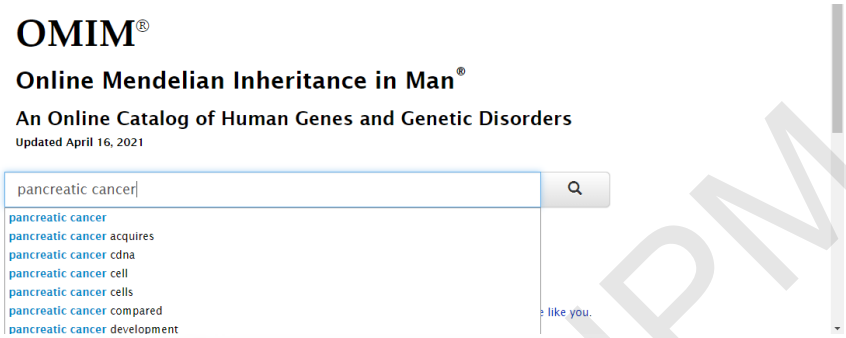


  - Click gene

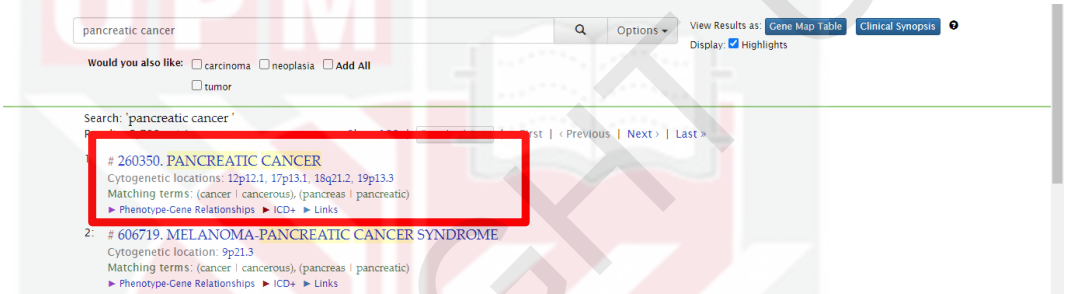
  - The result will be shown



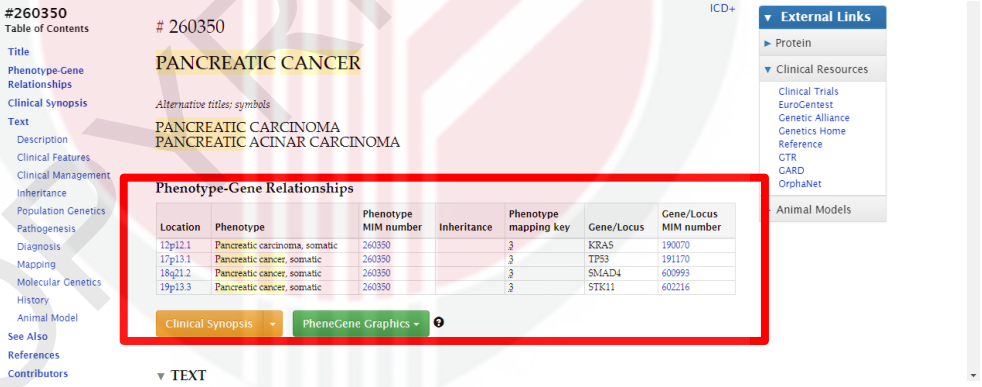
- **Online Mendelian Inheritance in Man (OMIM):** https://www.omim.org/
  - Enter the disease name (Pancreatic cancer) and click search



  - Choose pancreatic cancer



  - The pancreatic cancer-related gene will be shown



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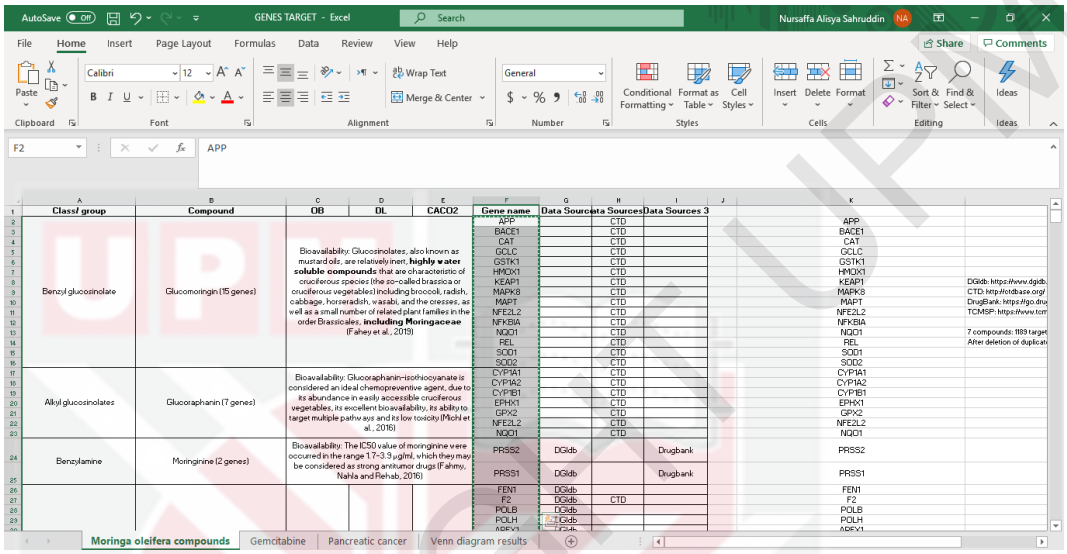
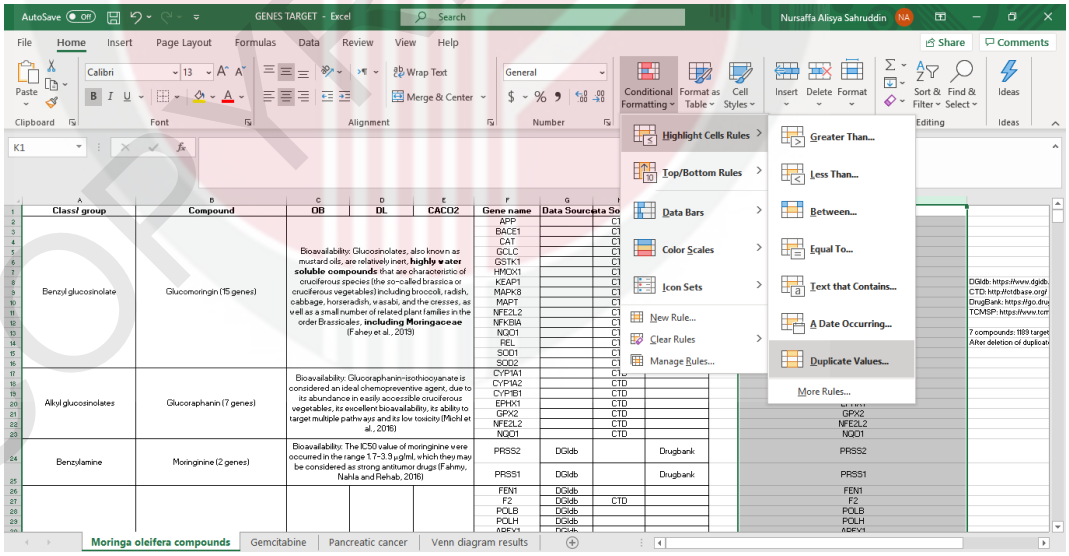
APPENDICES V

11.1 Screening potential targets of MO and GEM

- For *Moringa oleifera*, remove the duplicate target gene. Copy all the genes and paste into the new column.



- Select the new column, click conditional formatting, highlight cell rules and duplicate values



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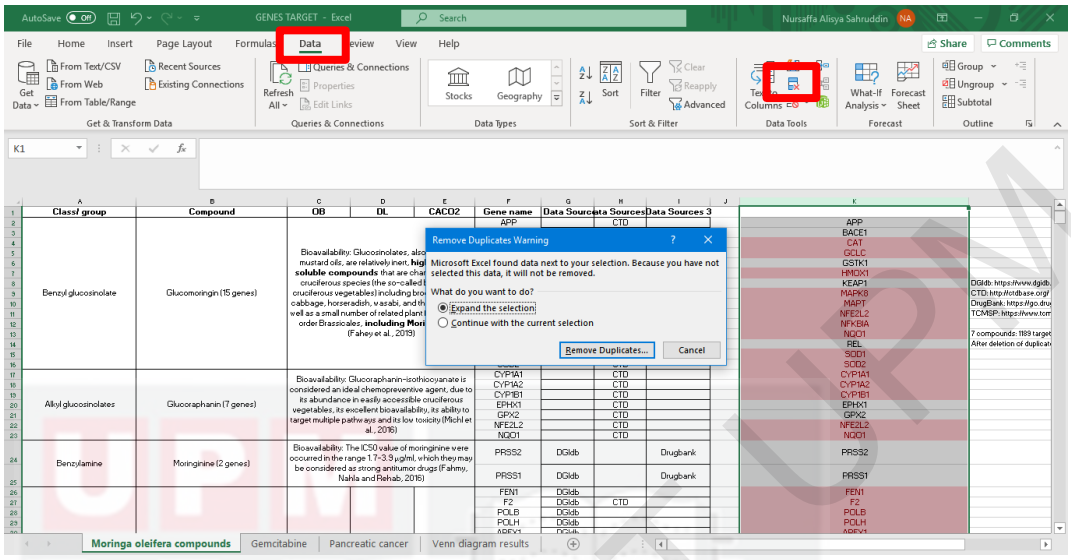
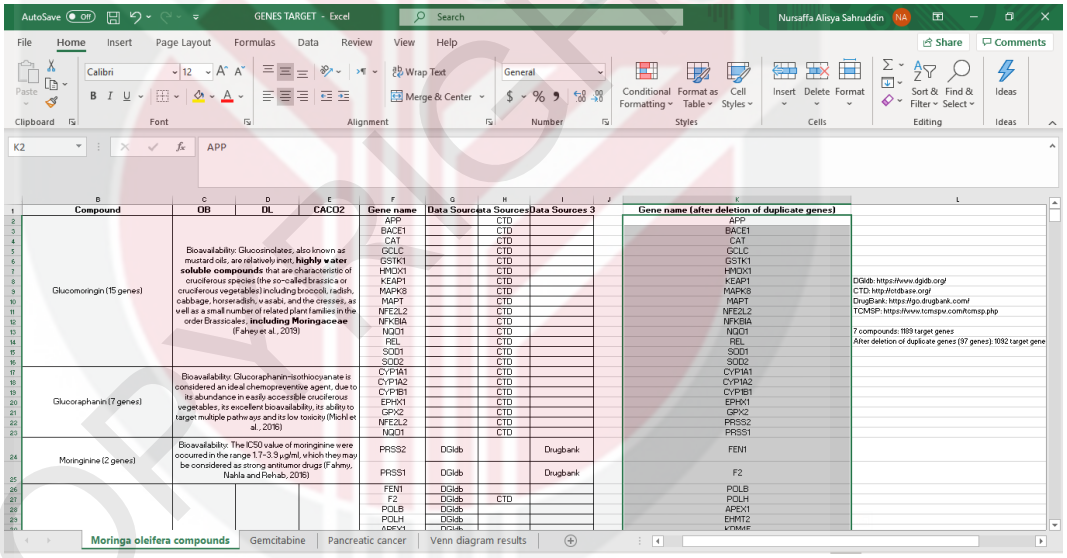
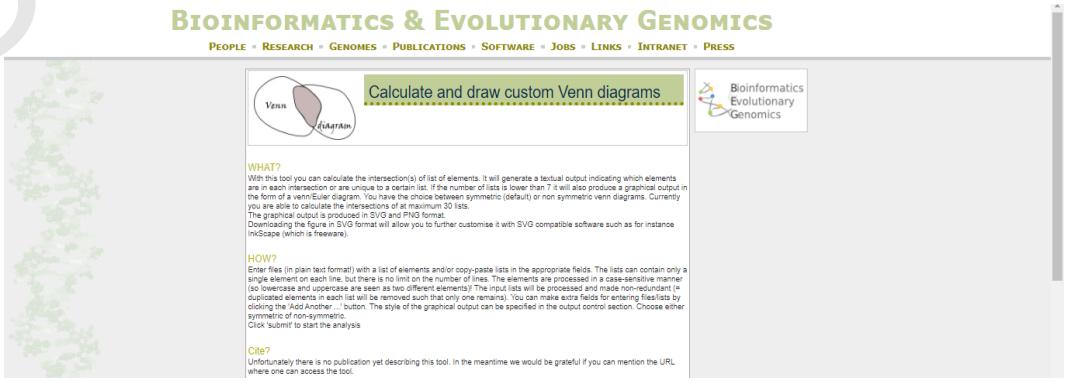
- Go to Data and click remove duplicate



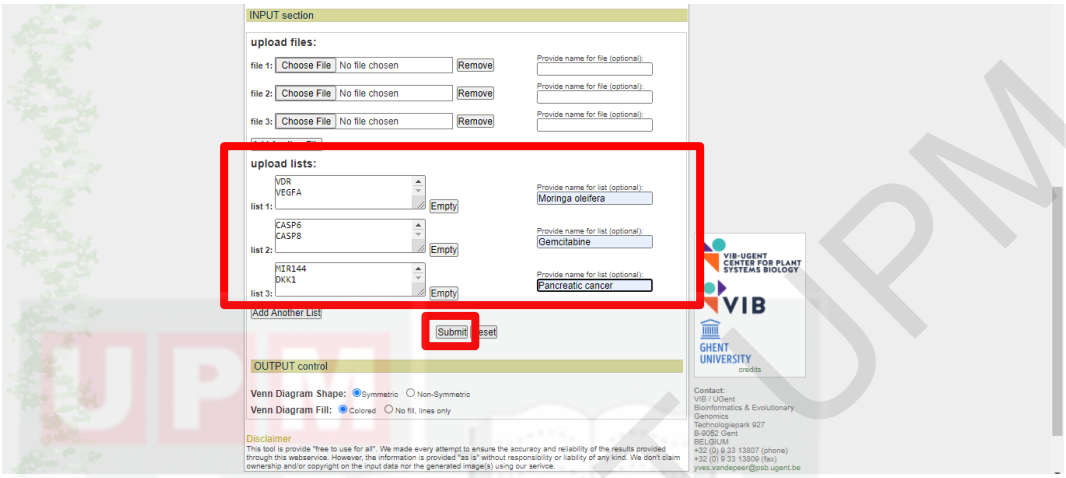
- Copy all the genes listed after remove the duplicate genes in Excel file



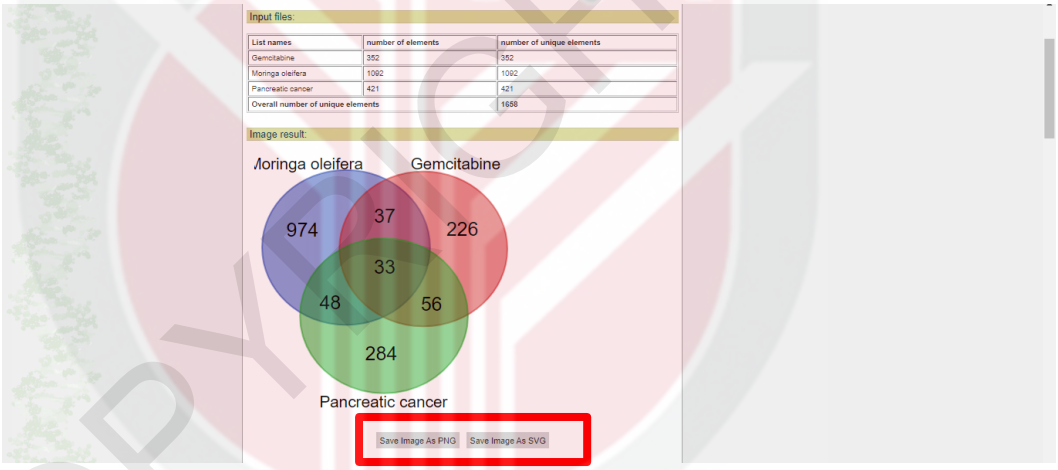
- Go to http://bioinformatics.psb.ugent.be/webtools/Venn/



- In the input section, paste all the target genes for Moringa oleifera, gemcitabine and pancreatic cancer in the separate list, provide name for each list and click submit



- The image and text result will be shown and can be downloaded



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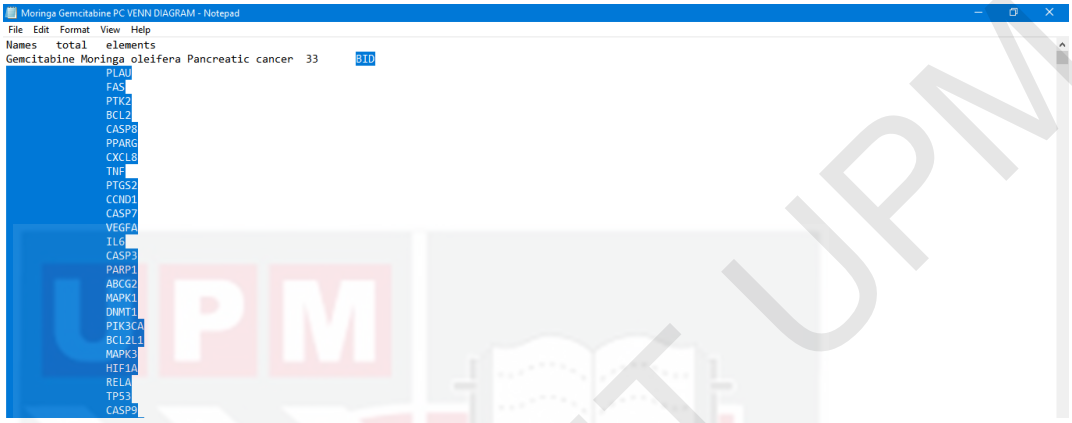
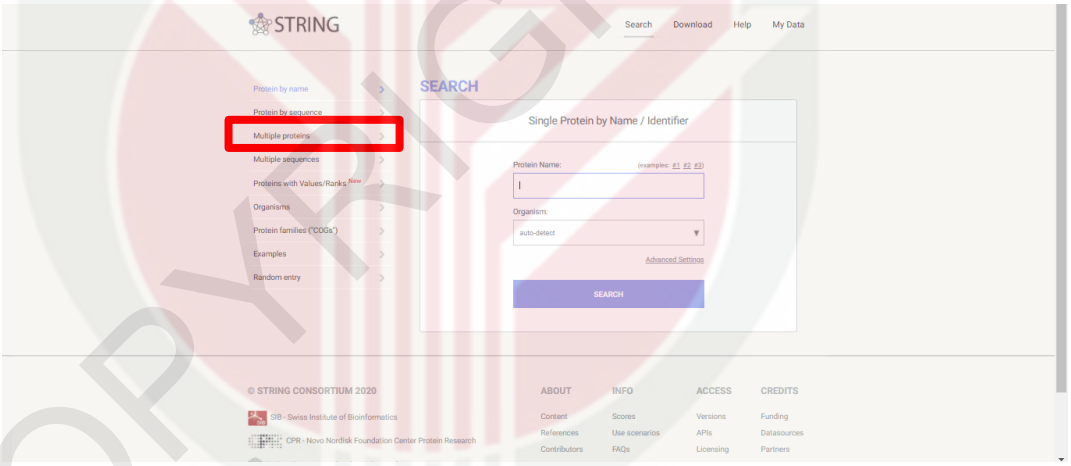
APPENDICES VI

11.1 Protein-protein interaction (PPI) network

- Copy the overlapping target genes from the text file



- Go to STRING Database 11.0 https://string-db.org/ and choose multiple protein



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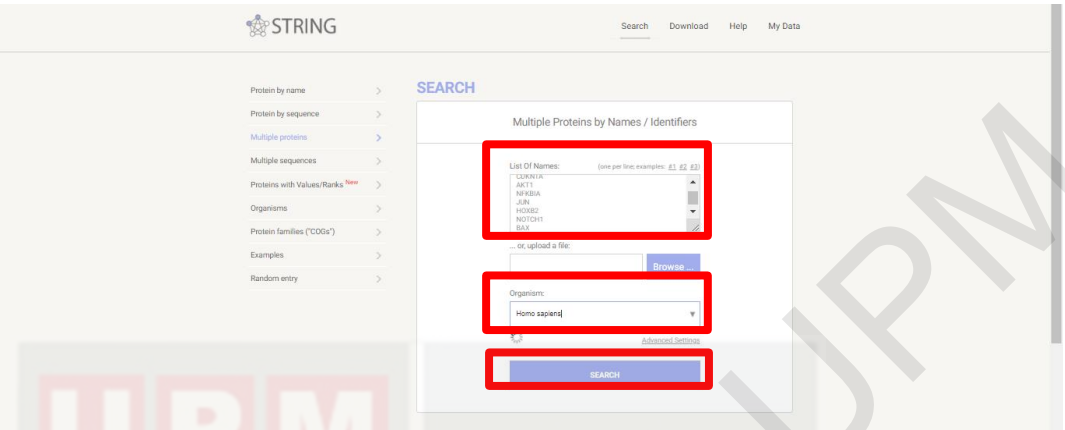
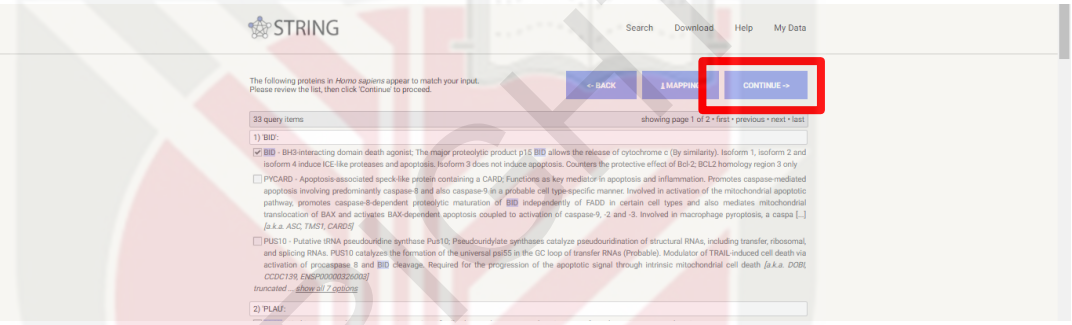
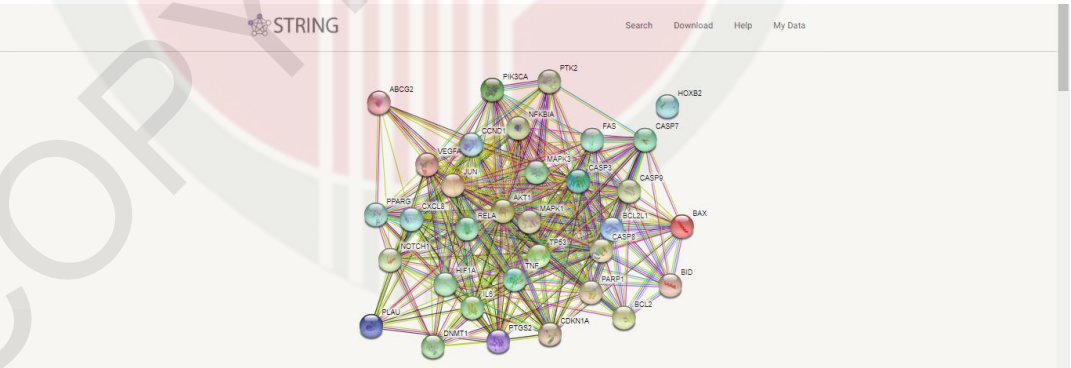
- Paste the overlapping genes, choose organism as Homo sapiens and click search



- Click continue



- Result of the network will be shown



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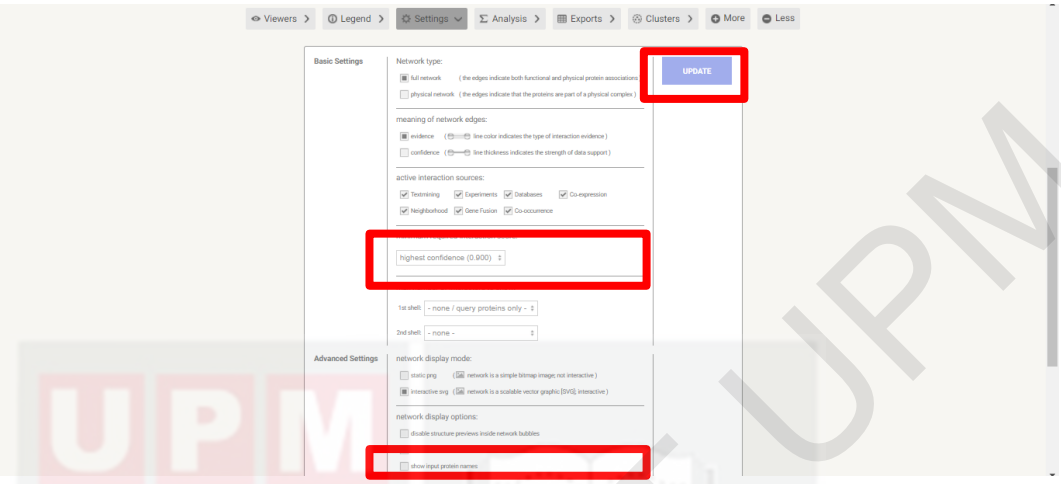
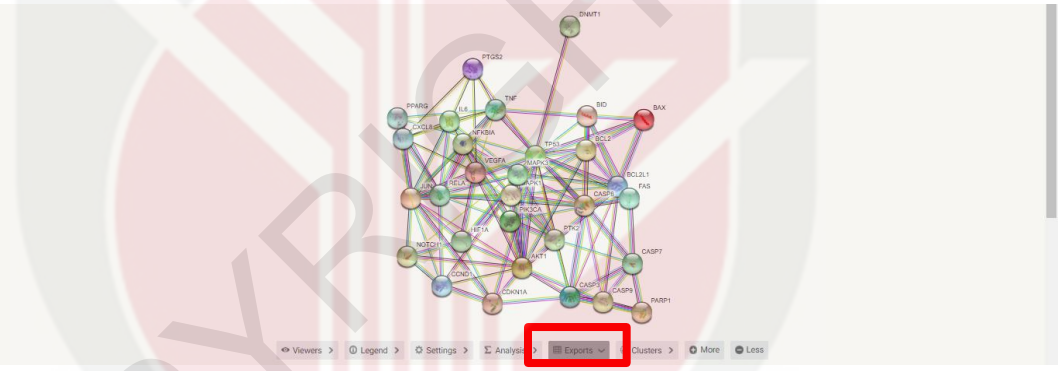
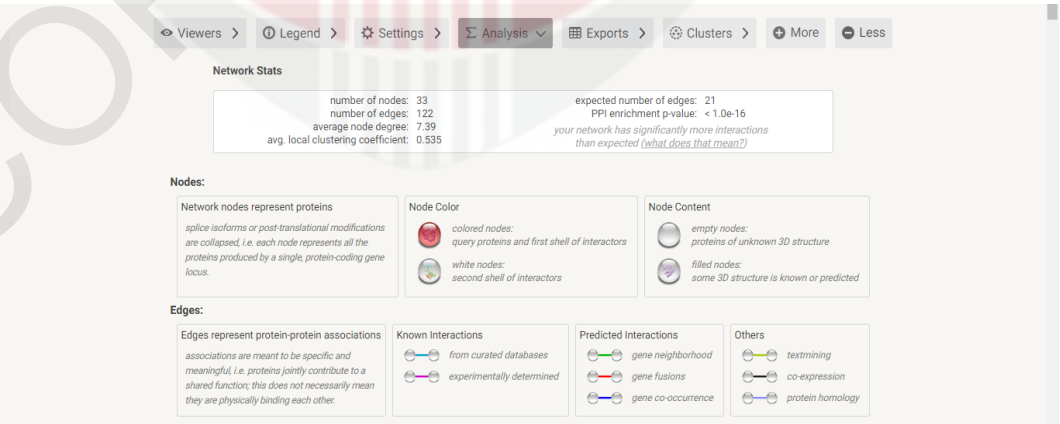
- For a better image of network, click setting, choose minimum required score as 0.900, hide disconnected nodes in the network and click update



- The result of the network will be updated. Network can be downloaded in image and Excel format by click the export



- The result of analysis and legend



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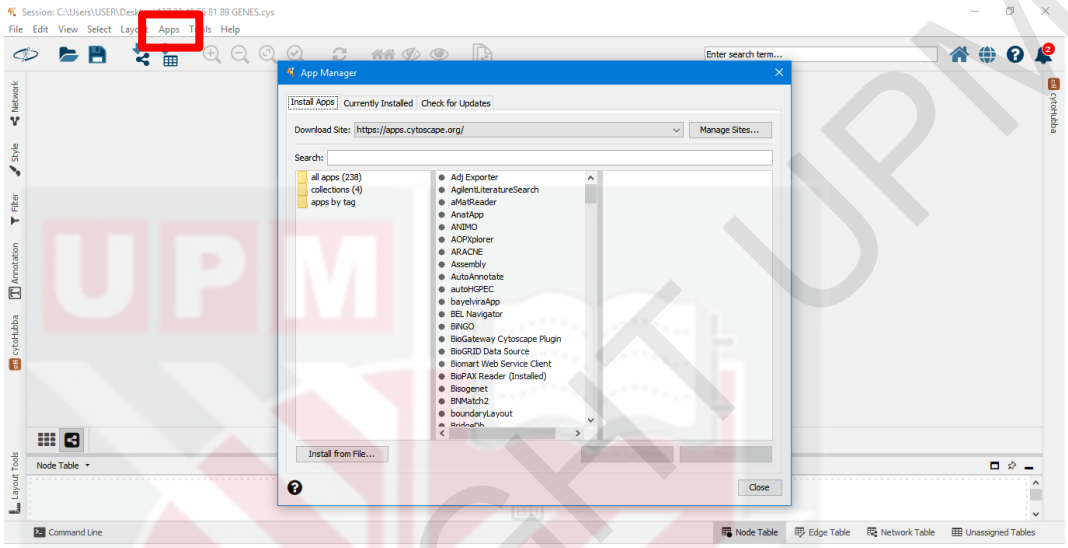
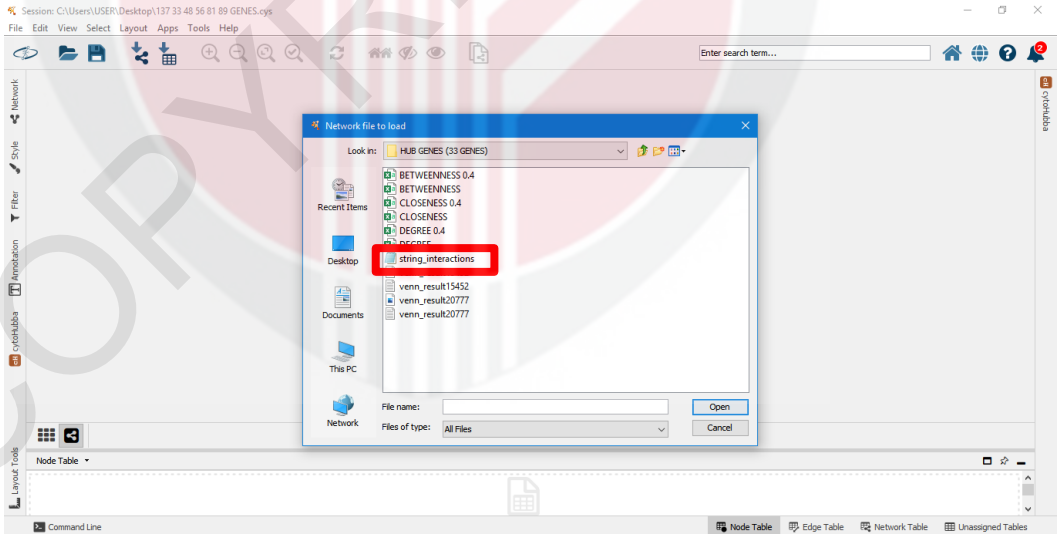
APPENDICE VII

11.1 Identification of hub genes

- Open Cytoscape. Go to app and click app manager to install cytoHubba to the software



- Import string_interactions file into the software. Click file → import → network from file



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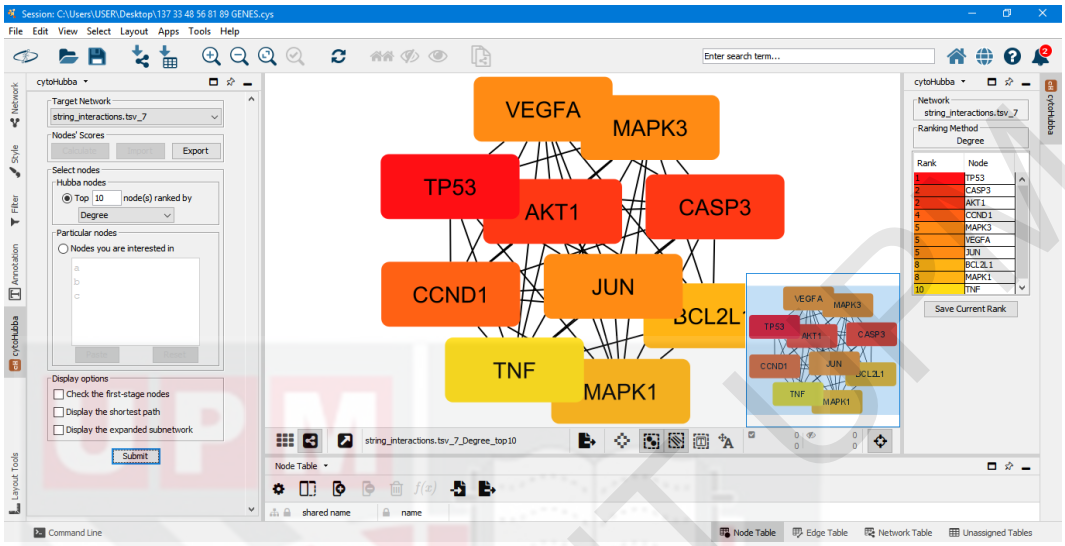
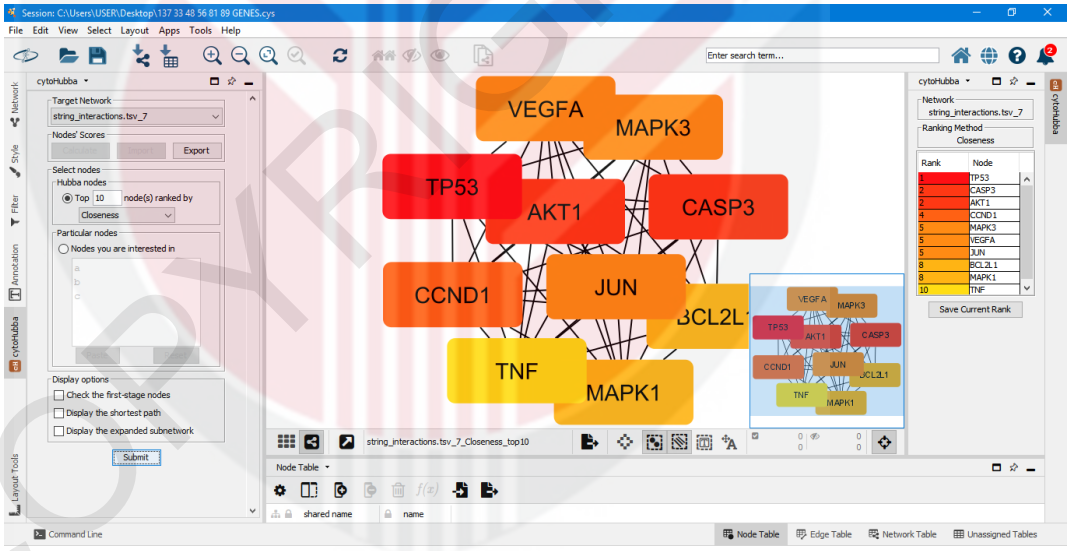
- Go to cytoHubba → calculate → select nodes as “top 10” → ranked by degree → submit. The result will be shown.

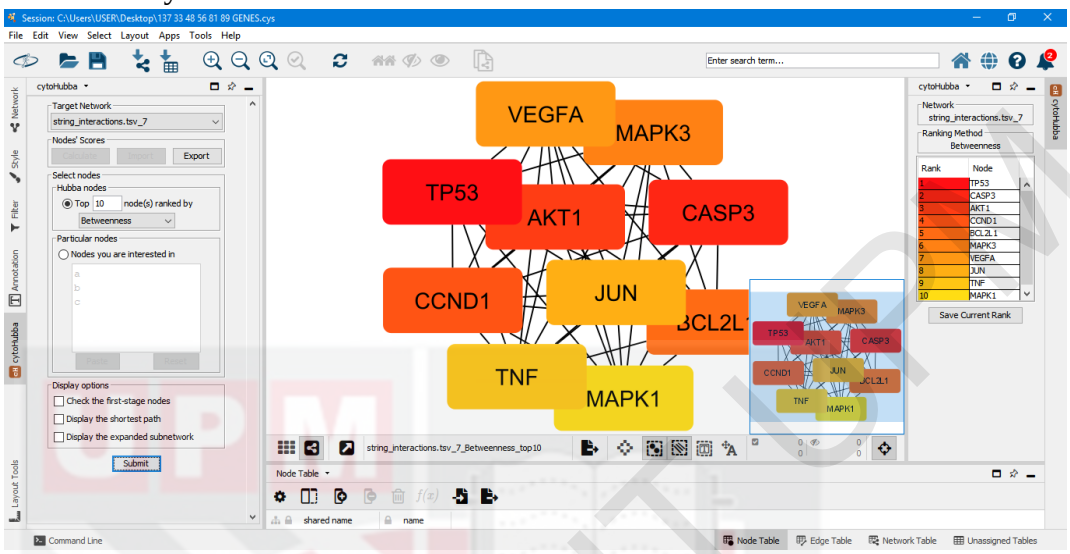


- Repeat the step. Go to cytoHubba → calculate → select nodes as “top 10” → ranked by closeness → submit. The result will be shown.

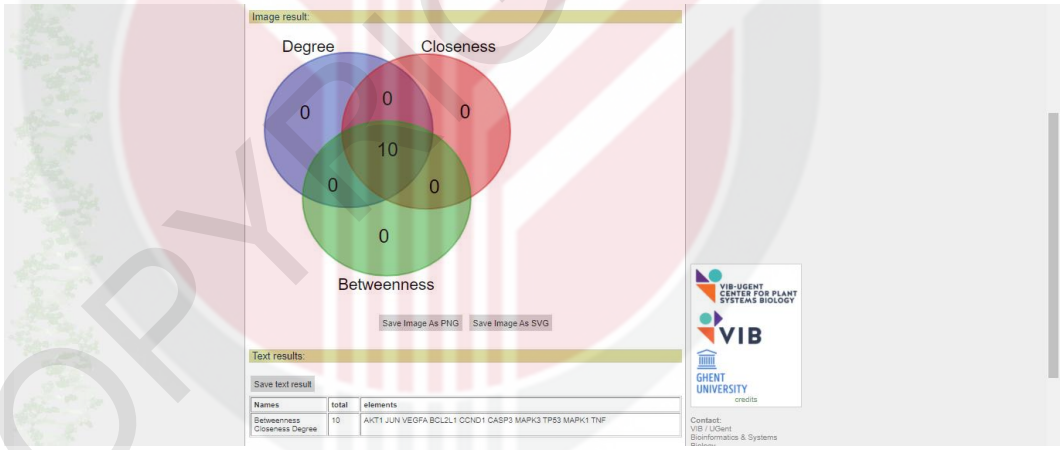


© COPYRIGHT

- Repeat the step. Go to cytoHubba → calculate → select nodes as “top 10” → ranked by betweenness → submit. The result will be shown.



- Find the intersection of the “top10” target genes. Put all the genes for the three calculations method into Bioinformatics and Evolutionary Genomics http://bioinformatics.psb.ugent.be/webtools/Venn/



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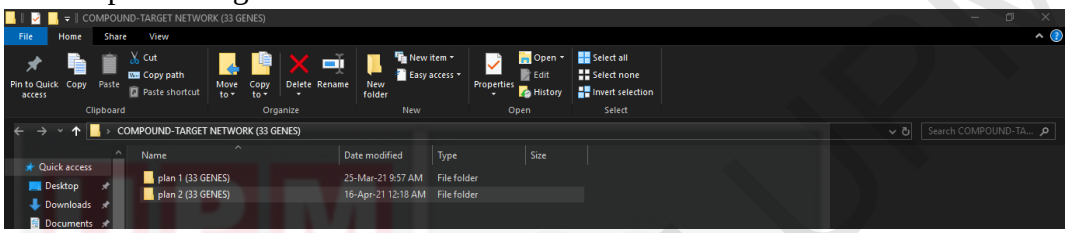
APPENDICES VIII

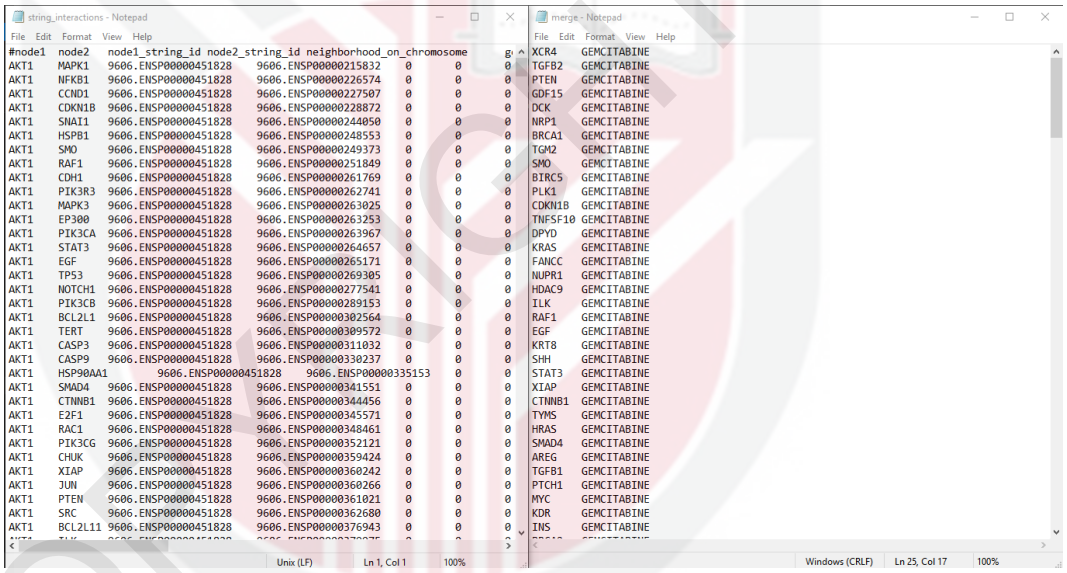
11.1 Compound-target network

11.1

- 2 plan files comprise of 5 individual files need to be prepared first before compound-target network can be constructed



i. Plan 1: string_interactions (from STRING database) and merge files



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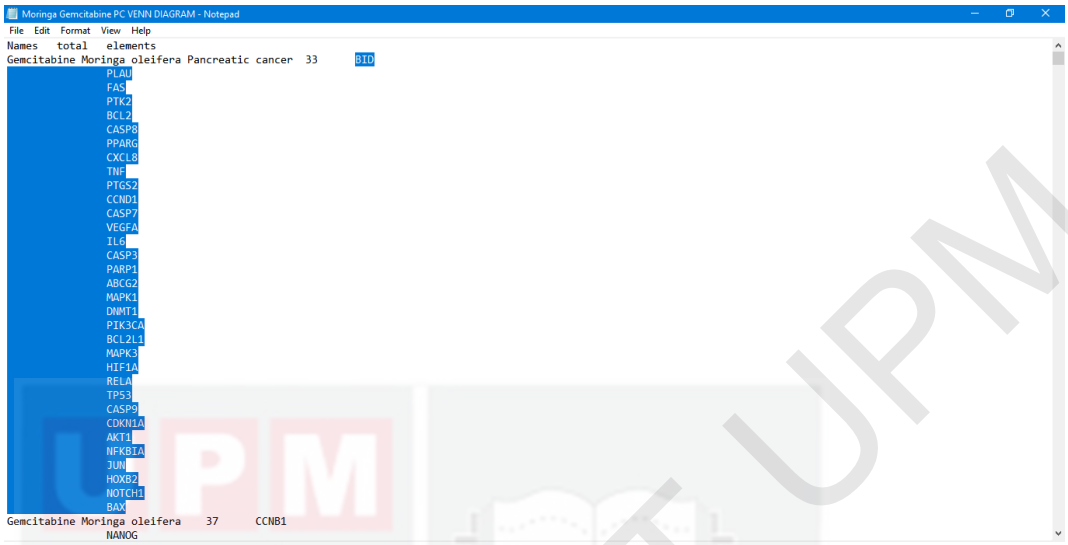
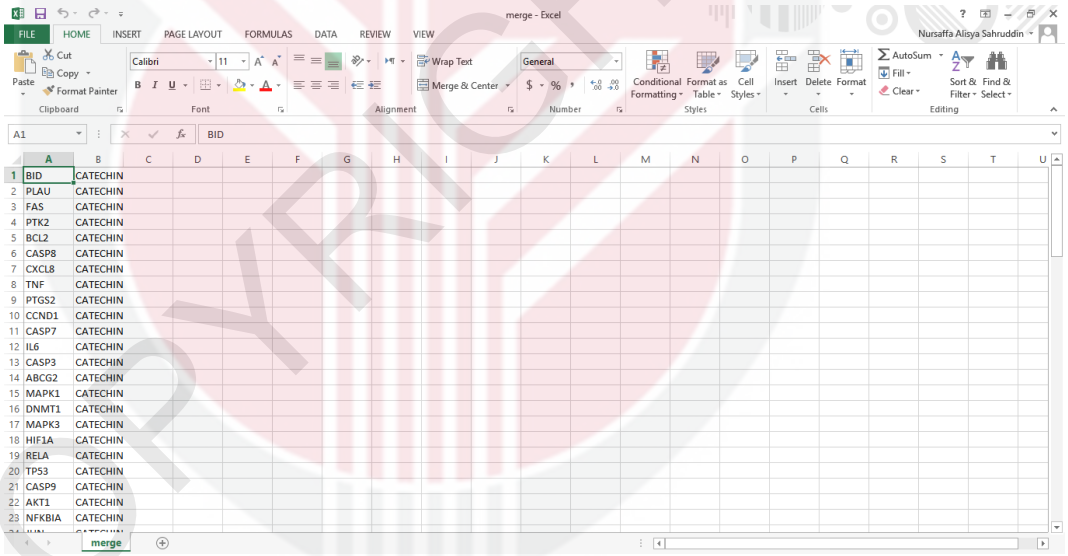
- Merge file is prepared by copy all the genes from the Venn diagram file



- Open Excel. Paste the genes in column A and put the compound name of the gene in column B. Save document as merge file in txt format



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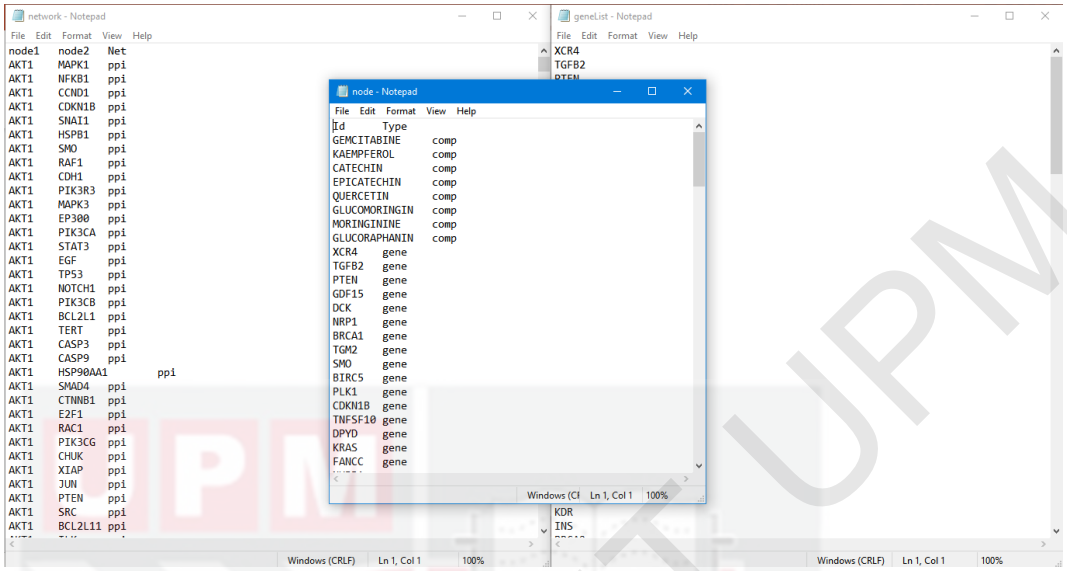
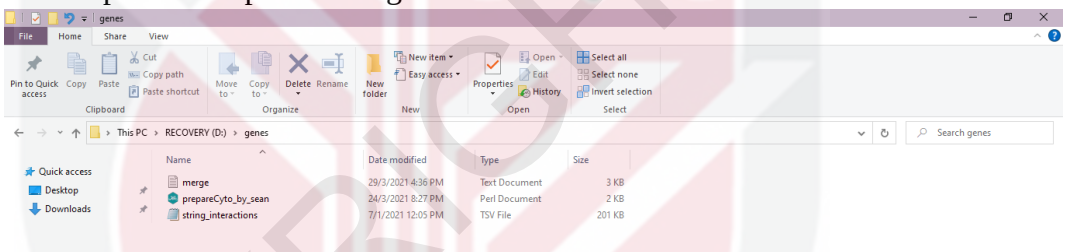
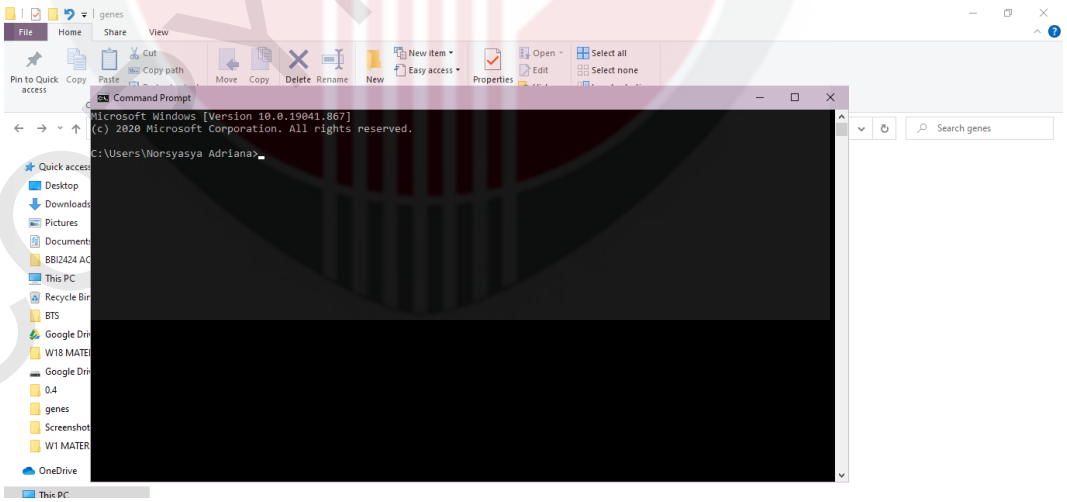
ii. Plan 2: network, geneList and node files in txt format



- Put plan 1 and perl file in gene file



- Open Command Prompt



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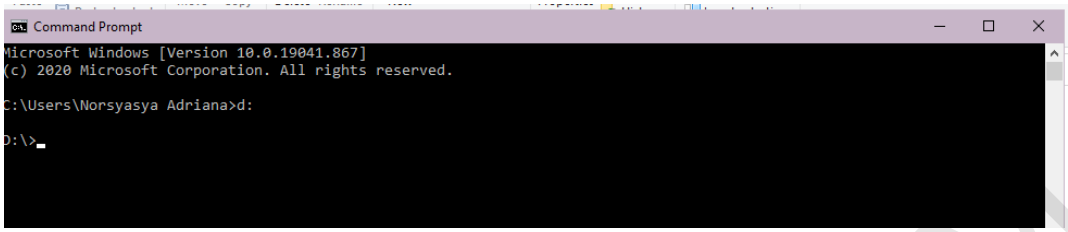
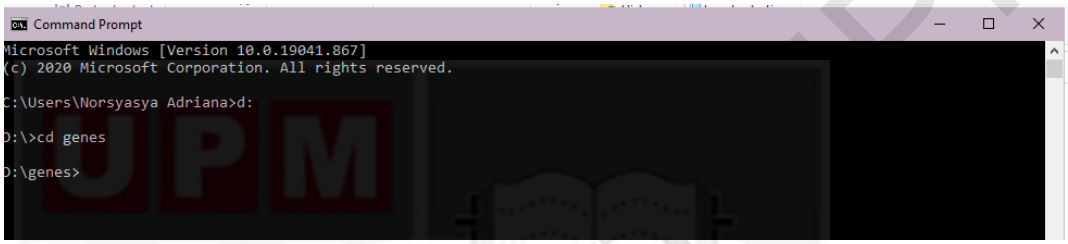
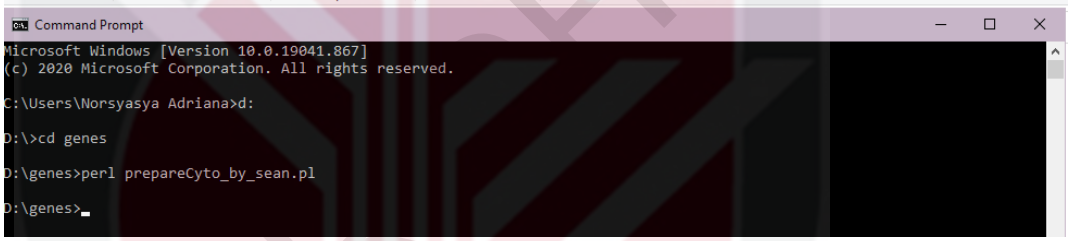
- Type d: and press enter



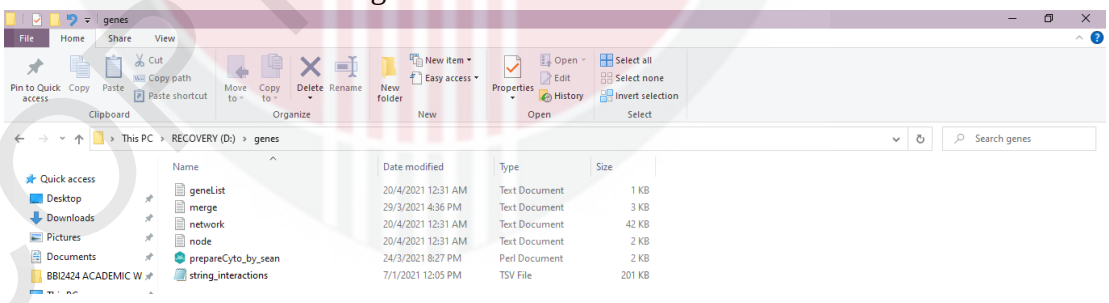
- Type cd genes and press enter



- Type perl prepareCyto_by_sean.pl and press enter



- geneList, network and node txt files will automatically be appeared. Check the result and add the missing data. Put the 3 files into a new folder title Plan 2.



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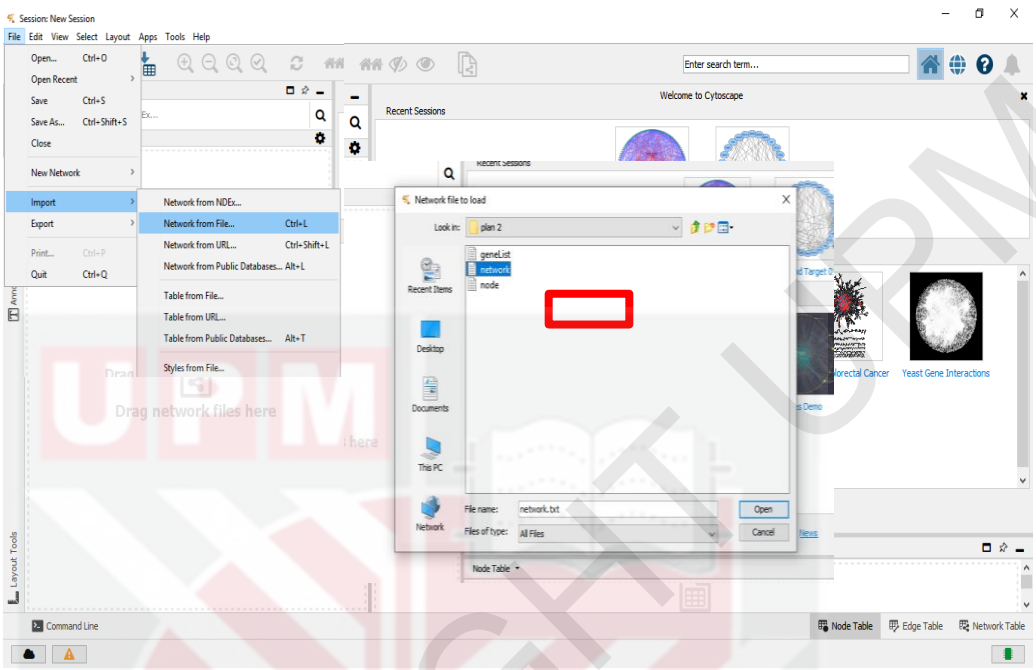
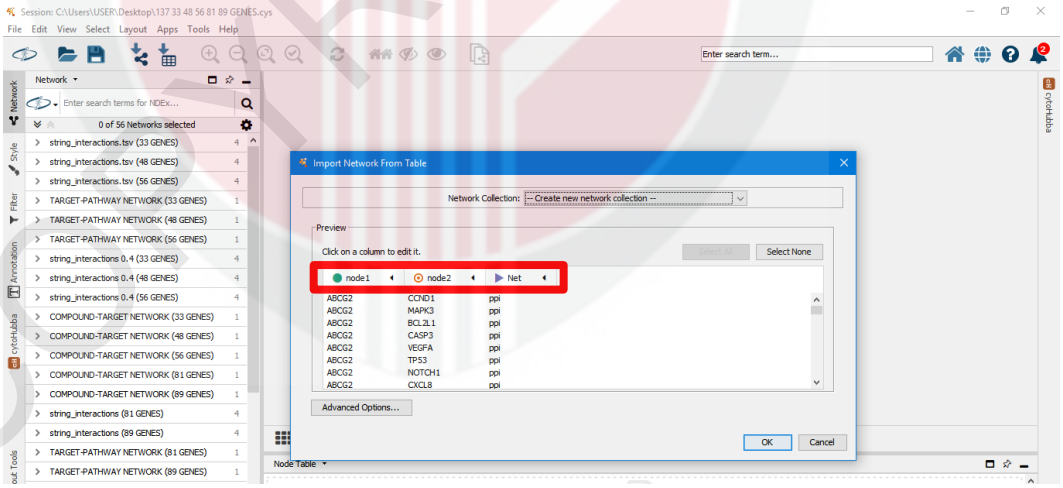
- Open Cytoscape and import the prepared file (network) into the software. Click file → import → network from file



- Change the name for each column: node 1 (source node), node 2 (target node), network (interaction type) → OK



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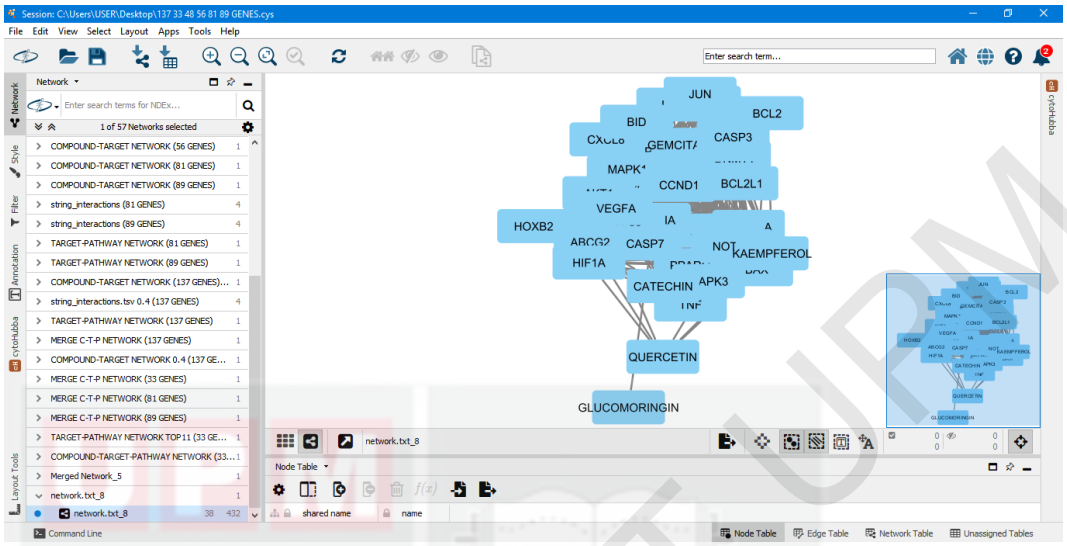
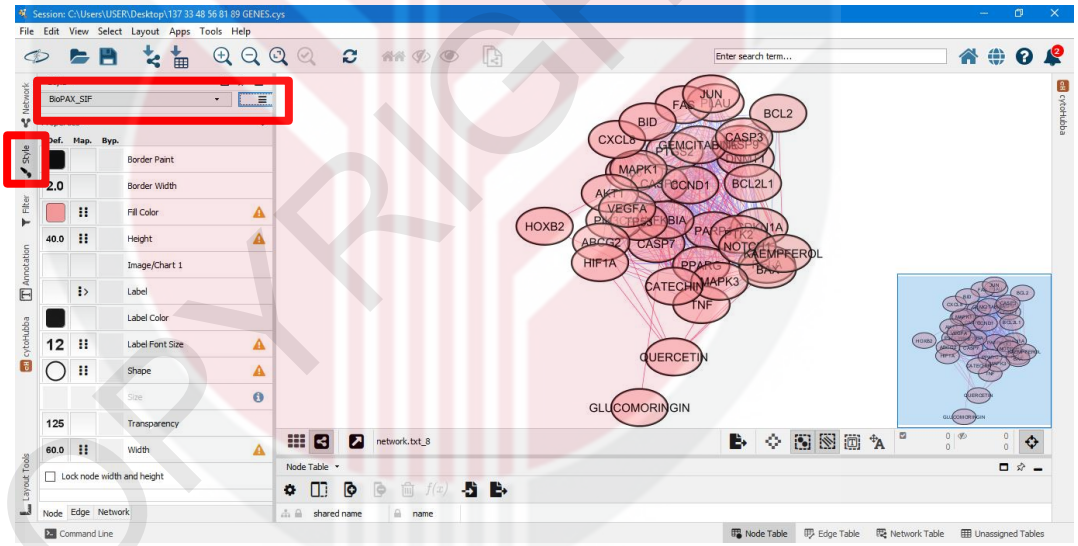
- The result of the network will be shown



- Change the style of the network according to own preference. Style → Default → BioPax_SIF



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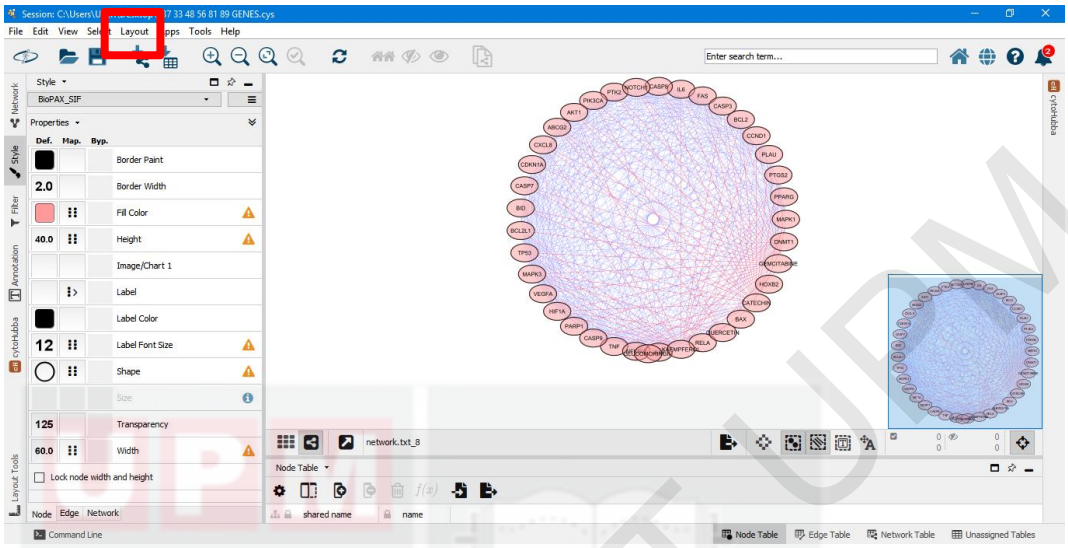
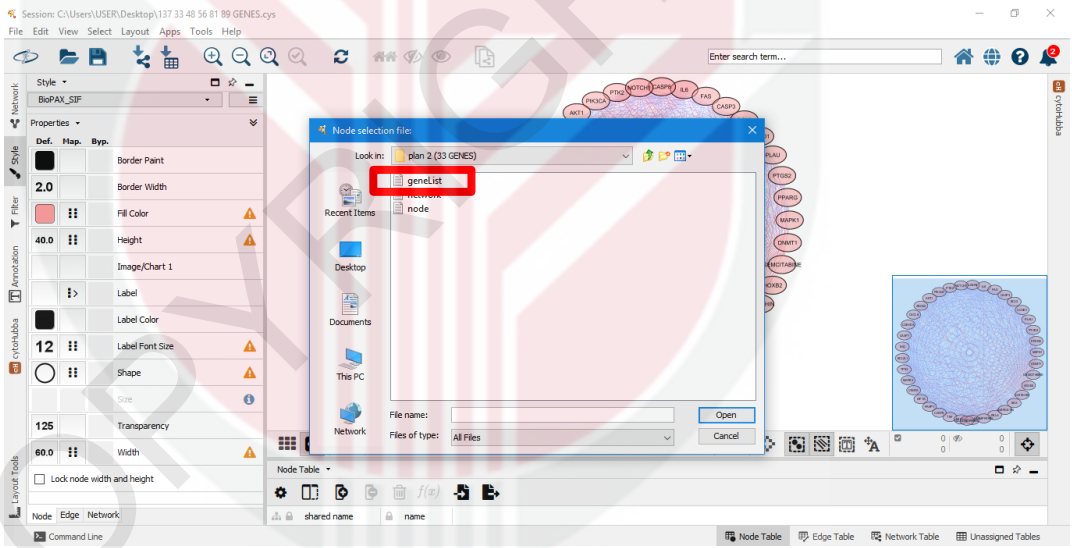
- Change the layout. Layout → circular layout



- Rearrange the compounds into the middle. Select → node → From ID list file → geneList



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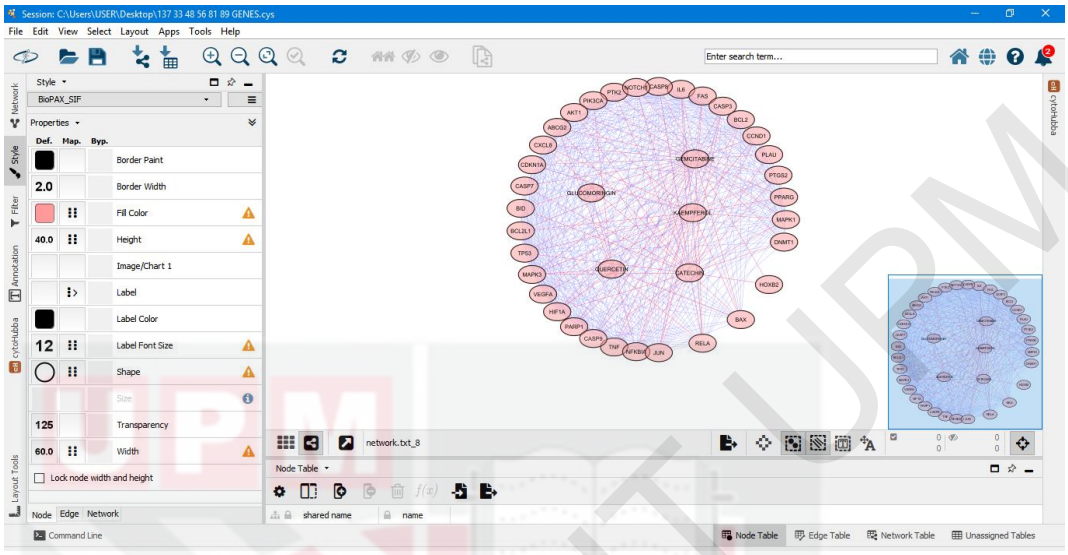
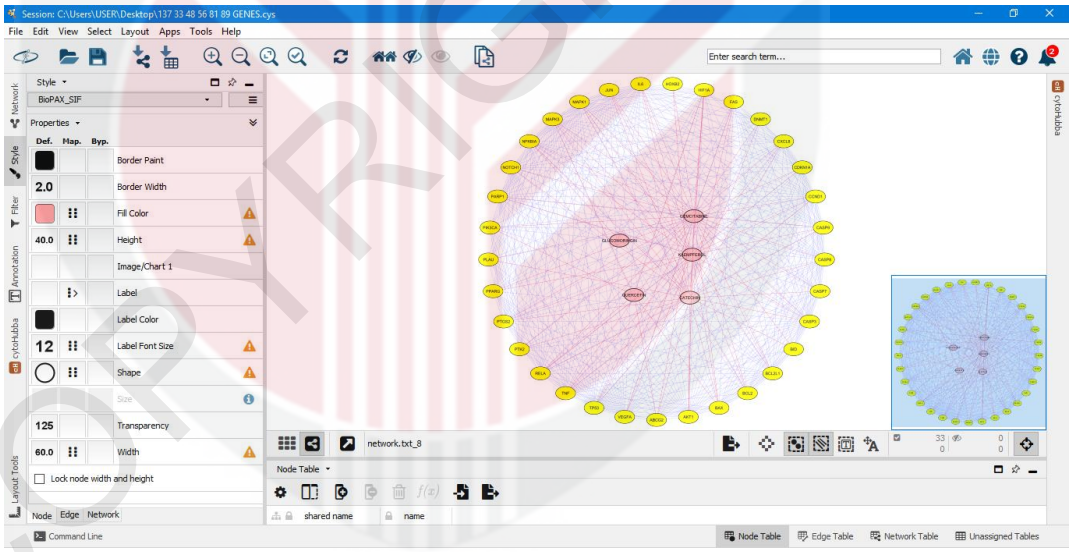
- The network will be shown like this after moving the compound into the middle



- Arrange the node in a complete circle. Layout → attribute circle layout → selected nodes only → name



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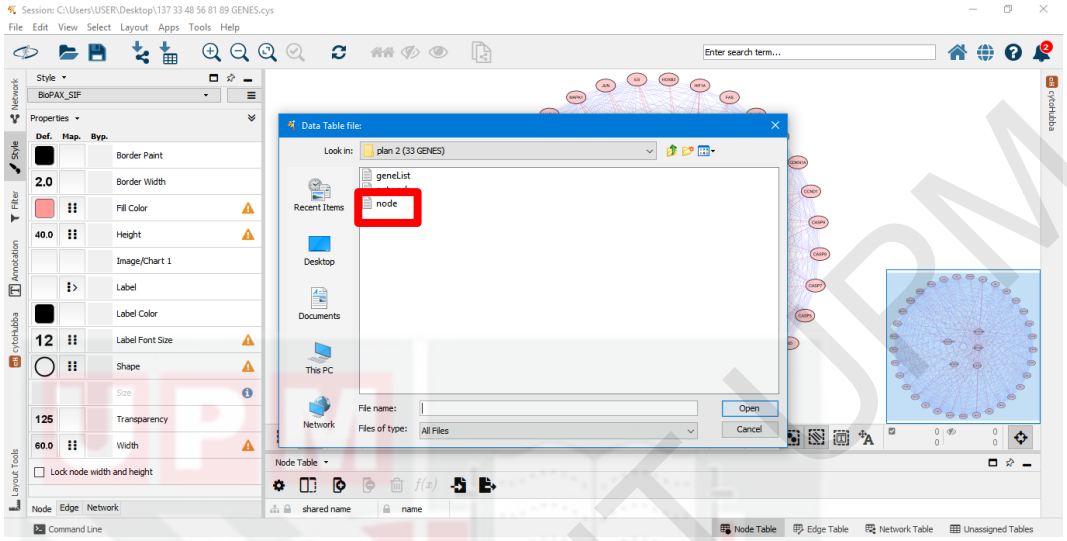
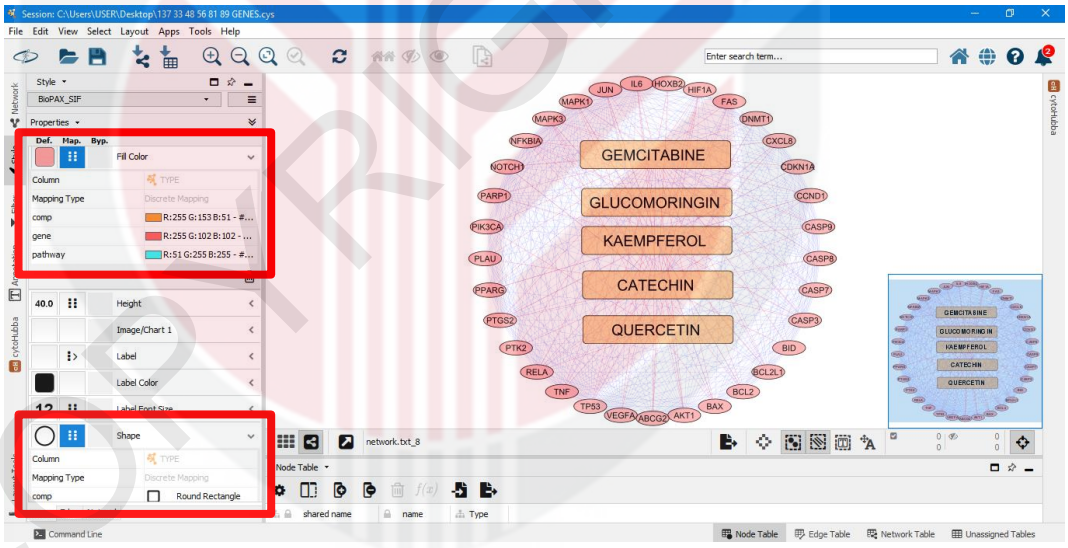
- To change the style of the node, import the node file to provide information for the Cytoscape. Import → Table from file → node



- Change the colour and shape of nodes of the compound and gene to differentiate them



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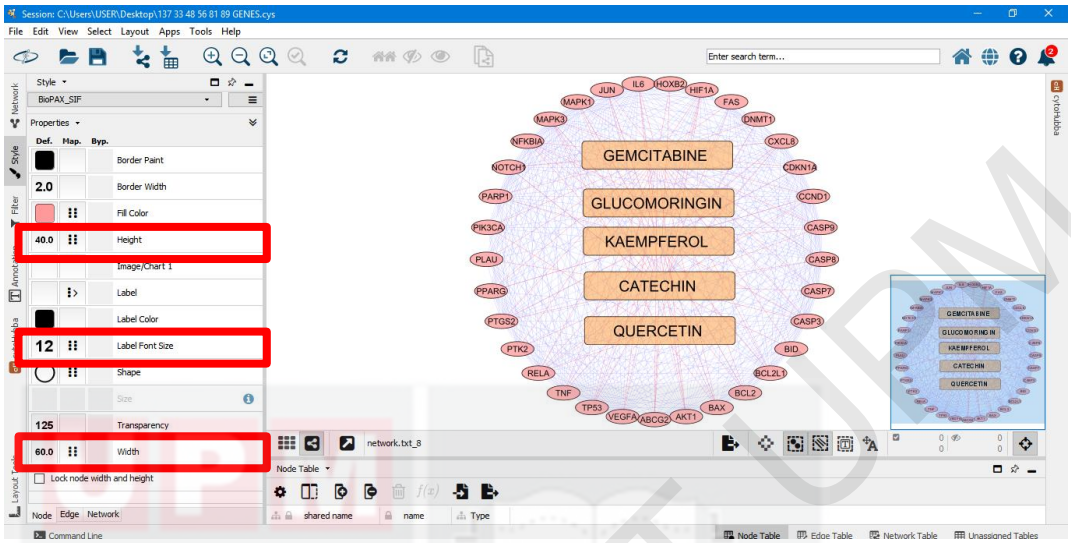
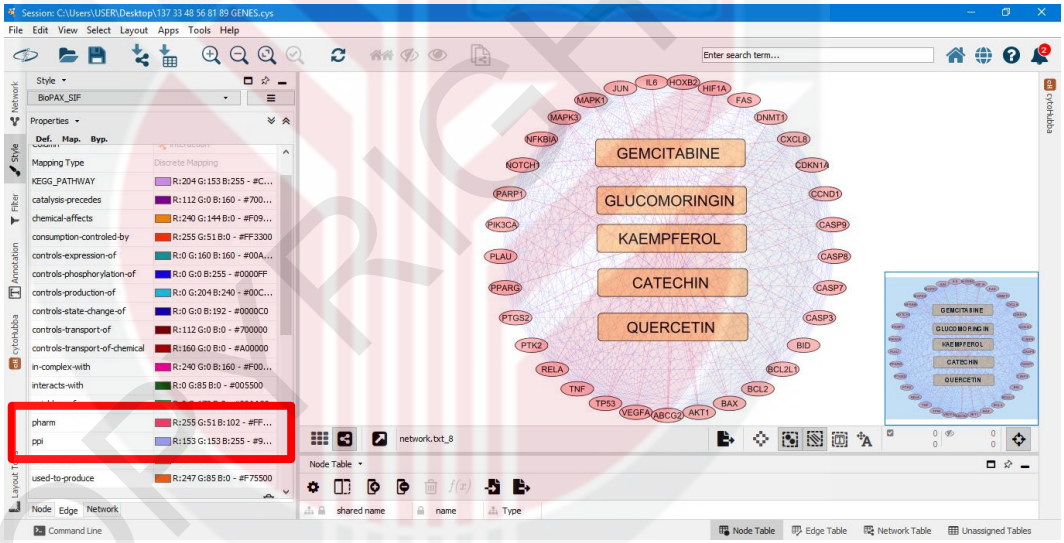
- The height and width of nodes as well as font size can also be changed



- Click Edge to change the colour of edges



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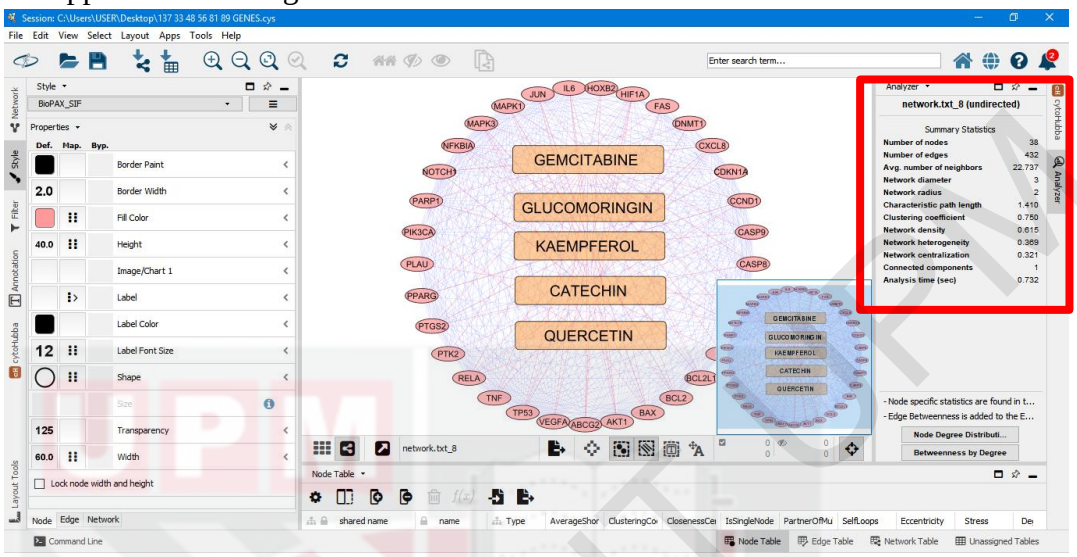
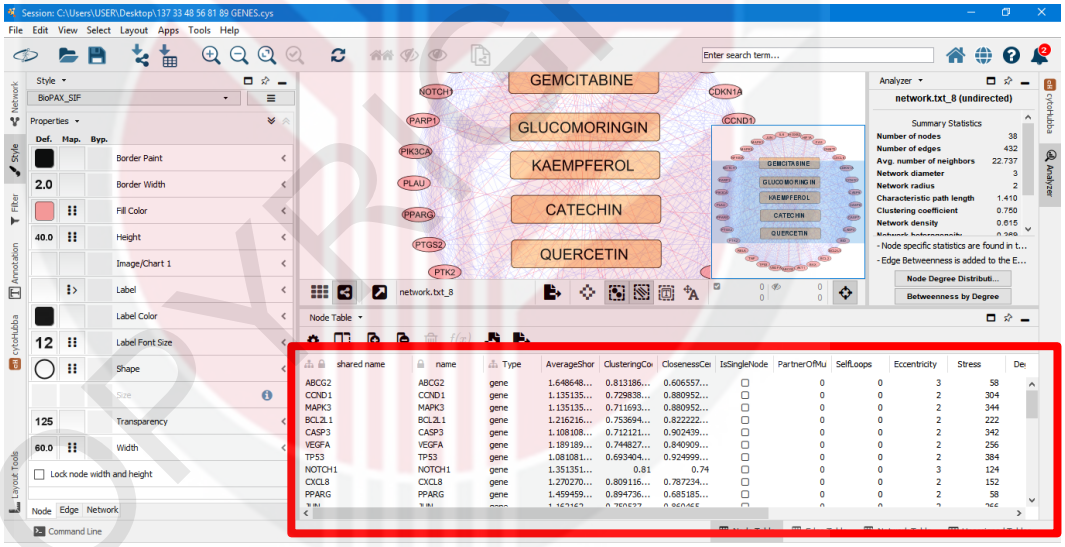
- For the result of analysis, click tool → analyze network. The result will be appeared on the right side



- The node information can be downloaded in excel file



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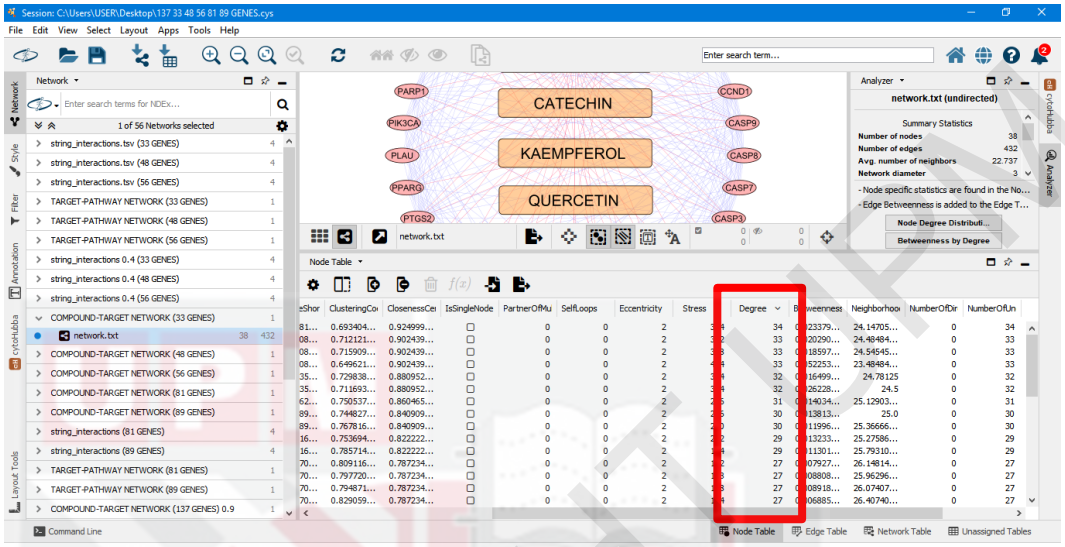
- From the node information, click degree to sort the value from higher to lower. Compound with more than average degree value is considered as hub compound. the average degree value can be obtained from the result of analysis



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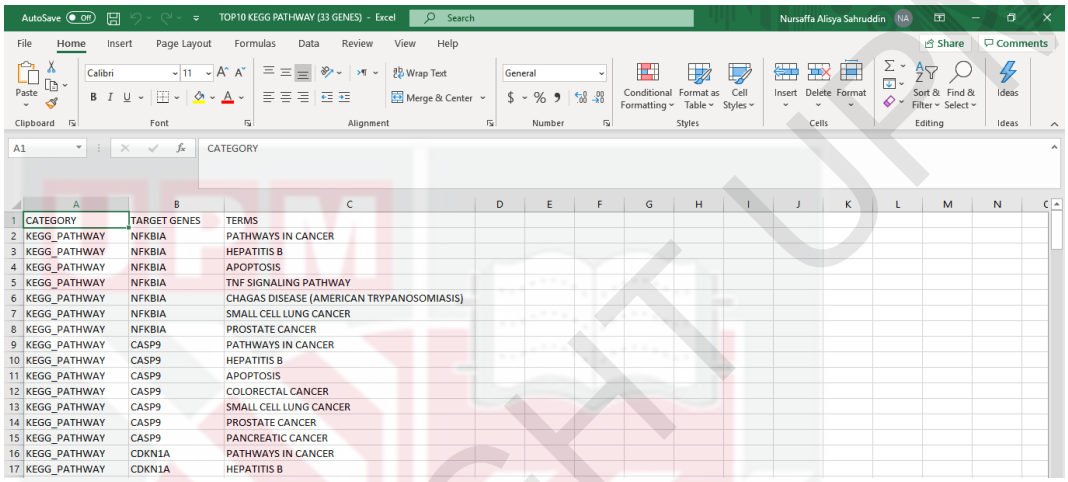
APPENDICES IX

11.1 Target-pathway network

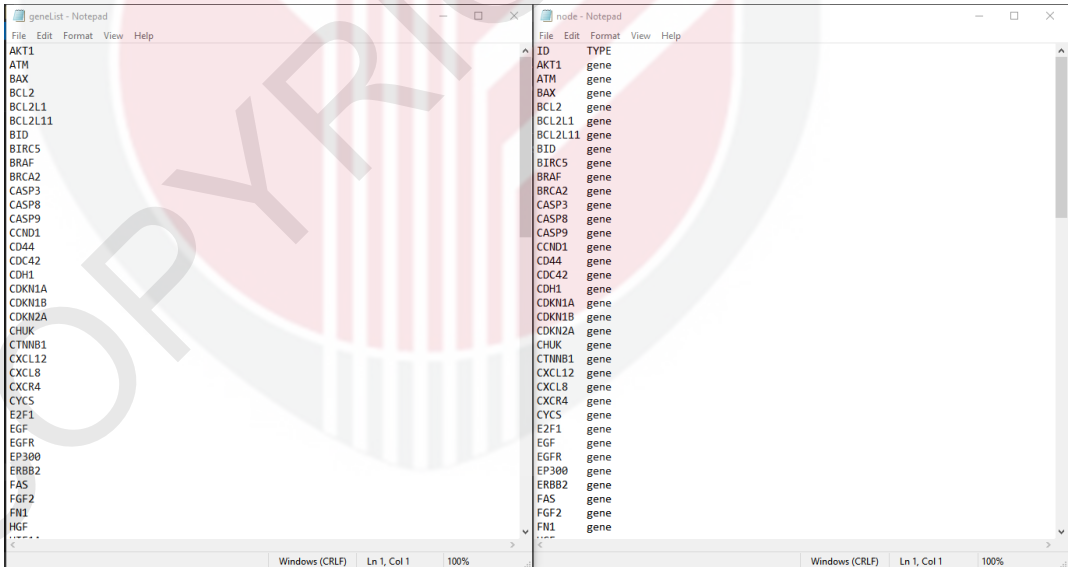
- 3 files need to be prepared first before target-pathway network can be constructed

i. Top 10 KEGG pathway in Excel file



	A	B	C
1	CATEGORY	TARGET GENES	TERMS
2	KEGG_PATHWAY	NFKBIA	PATHWAYS IN CANCER
3	KEGG_PATHWAY	NFKBIA	HEPATITIS B
4	KEGG_PATHWAY	NFKBIA	APOPTOSIS
5	KEGG_PATHWAY	NFKBIA	TNF SIGNALING PATHWAY
6	KEGG_PATHWAY	NFKBIA	CHAGAS DISEASE (AMERICAN TRYPANOSOMIASIS)
7	KEGG_PATHWAY	NFKBIA	SMALL CELL LUNG CANCER
8	KEGG_PATHWAY	NFKBIA	PROSTATE CANCER
9	KEGG_PATHWAY	CASP9	PATHWAYS IN CANCER
10	KEGG_PATHWAY	CASP9	HEPATITIS B
11	KEGG_PATHWAY	CASP9	APOPTOSIS
12	KEGG_PATHWAY	CASP9	COLORECTAL CANCER
13	KEGG_PATHWAY	CASP9	SMALL CELL LUNG CANCER
14	KEGG_PATHWAY	CASP9	PROSTATE CANCER
15	KEGG_PATHWAY	CASP9	PANCREATIC CANCER
16	KEGG_PATHWAY	CDKN1A	PATHWAYS IN CANCER
17	KEGG_PATHWAY	CDKN1A	HEPATITIS B

ii. geneList and node file in txt format



geneList - Notepad

```
AKT1
ATM
BAX
BCL2
BCL2L1
BCL2L11
BID
BIRC5
BRAF
BRCA2
CASP3
CASP8
CASP9
CCND1
CD44
CDC42
CDH1
CDKN1A
CDKN1B
CDKN2A
CHUK
CTNNB1
CXCL12
CXCL8
CXCR4
CYCS
E2F1
EGF
EGFR
EP300
ERBB2
FAS
FGF2
FN1
HGF
```

node - Notepad

ID	TYPE
AKT1	gene
ATM	gene
BAX	gene
BCL2	gene
BCL2L1	gene
BCL2L11	gene
BID	gene
BIRC5	gene
BRAF	gene
BRCA2	gene
CASP3	gene
CASP8	gene
CASP9	gene
CCND1	gene
CD44	gene
CDC42	gene
CDH1	gene
CDKN1A	gene
CDKN1B	gene
CDKN2A	gene
CHUK	gene
CTNNB1	gene
CXCL12	gene
CXCL8	gene
CXCR4	gene
CYCS	gene
E2F1	gene
EGF	gene
EGFR	gene
EP300	gene
ERBB2	gene
FAS	gene
FGF2	gene
FN1	gene

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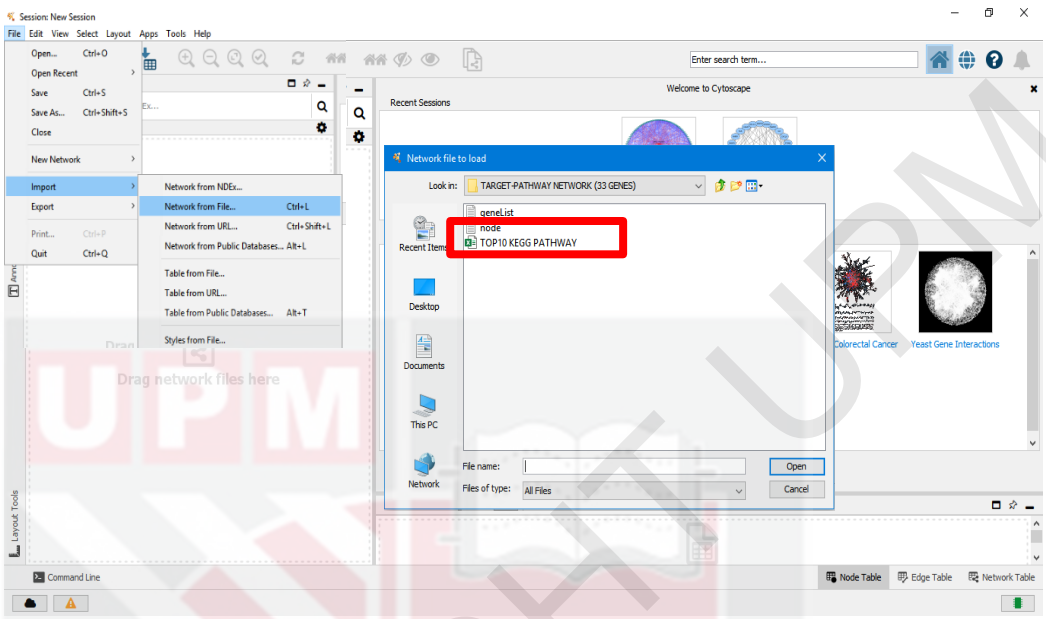
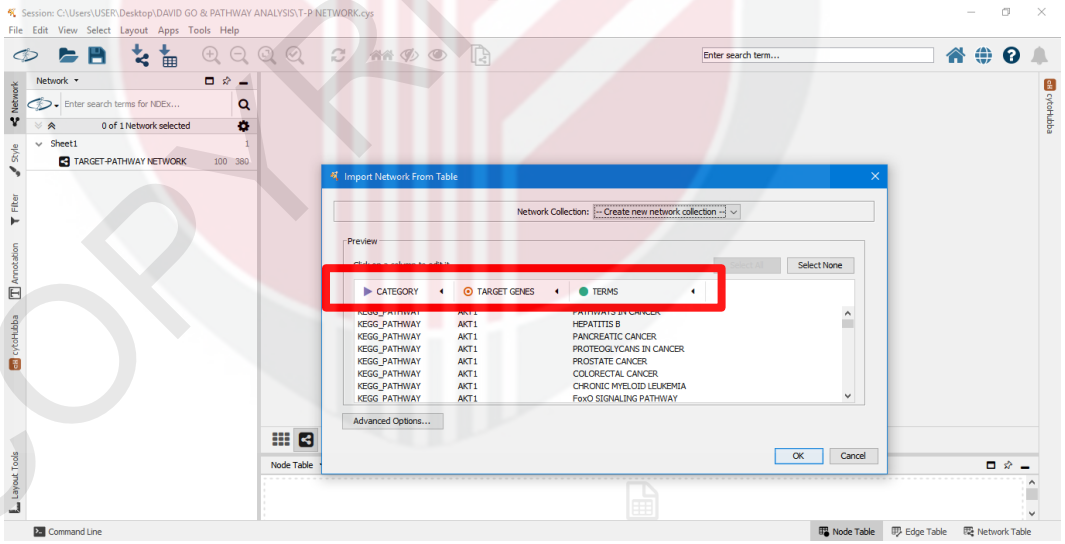
- Open Cytoscape and import the prepared file (network) into the software. Click file → import → network from file



- Change the name for each column: category (interaction) > target genes (target node) > terms (source node) > OK



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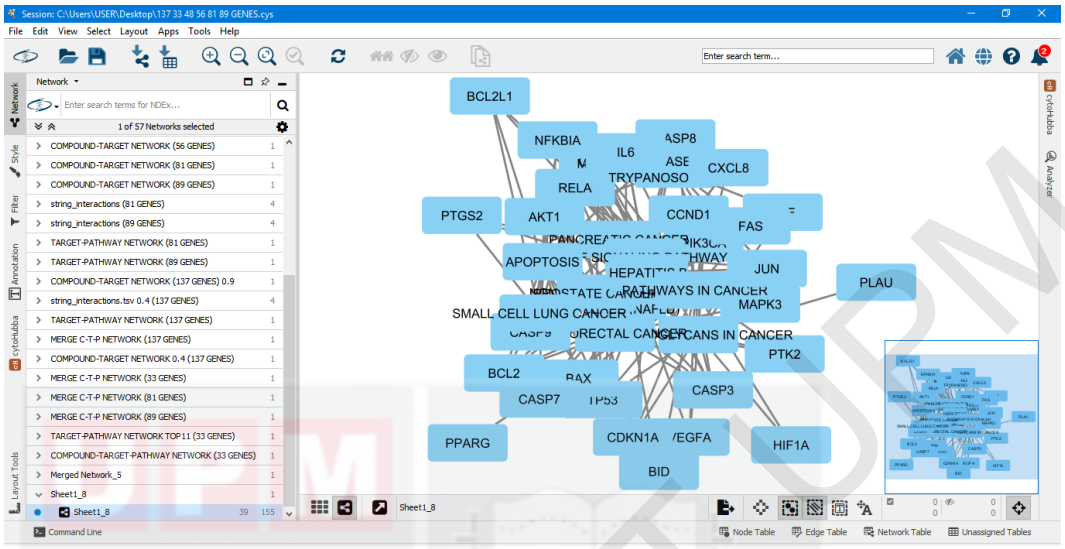
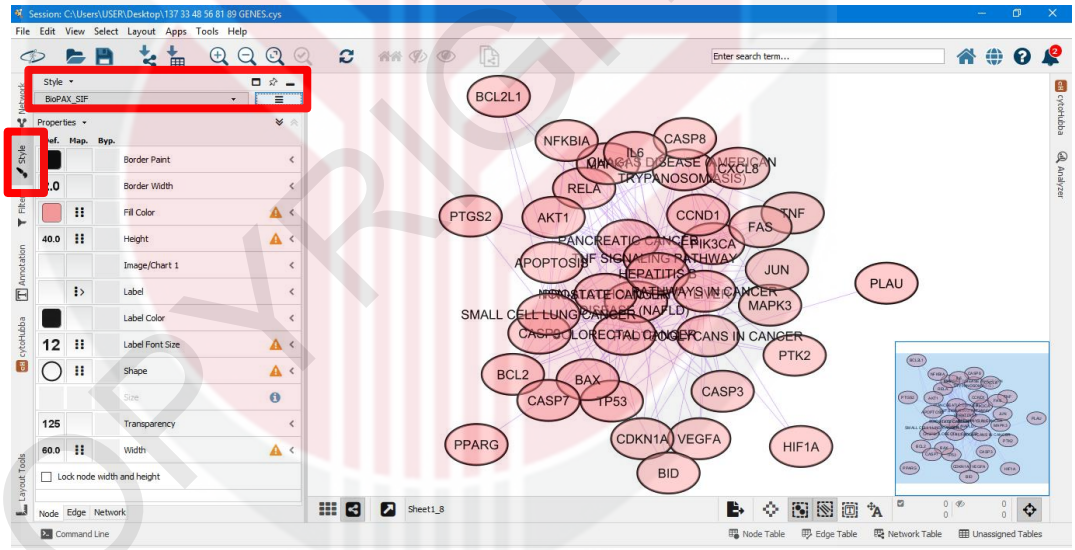
- The result of the network will be shown



- Change the style of the network according to own preference. Style → Default → BioPax_SIF



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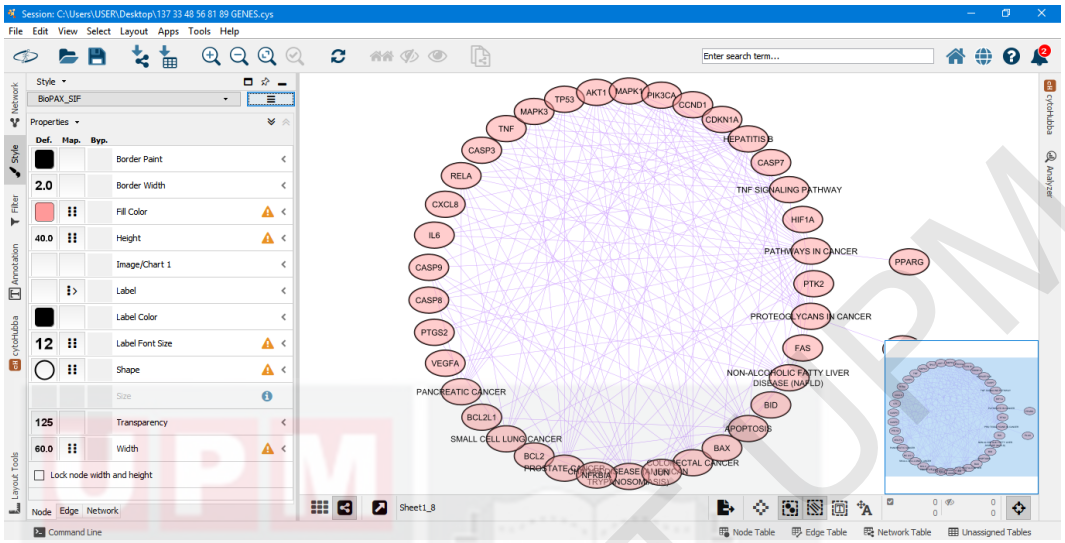
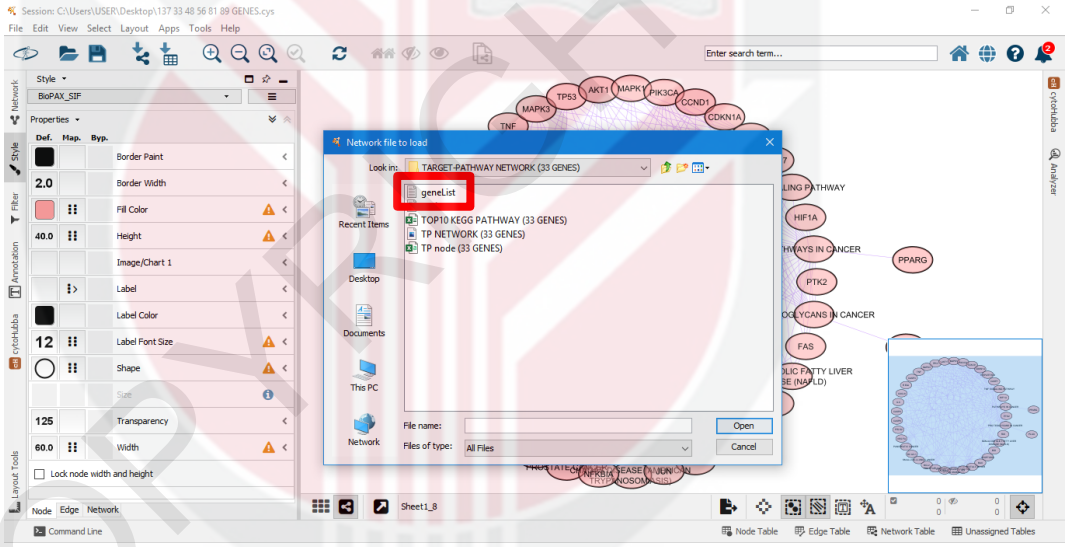
- Change the layout. Layout → circular layout



- Rearrange the pathways. Select → node → From ID list file → geneList



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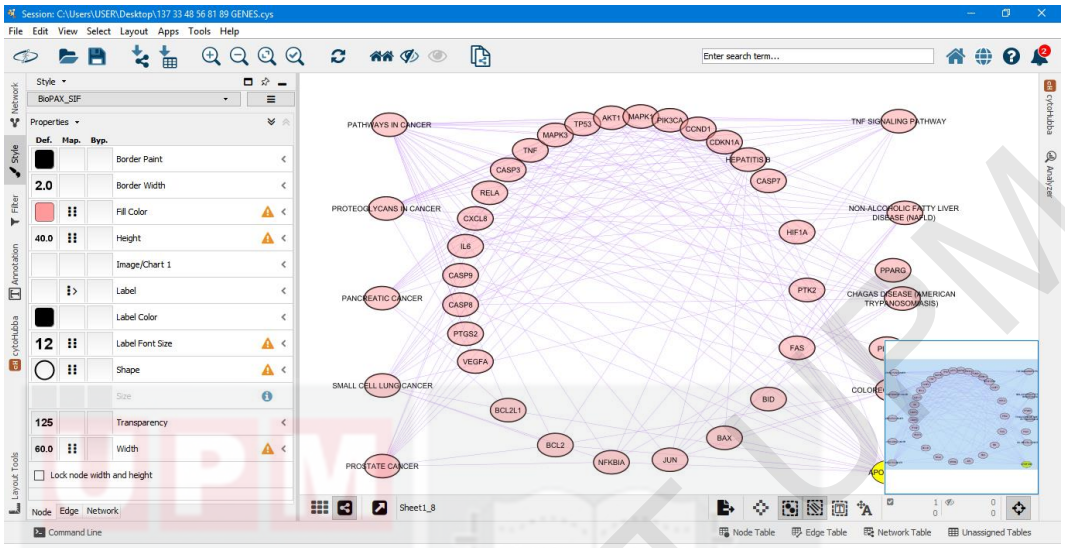
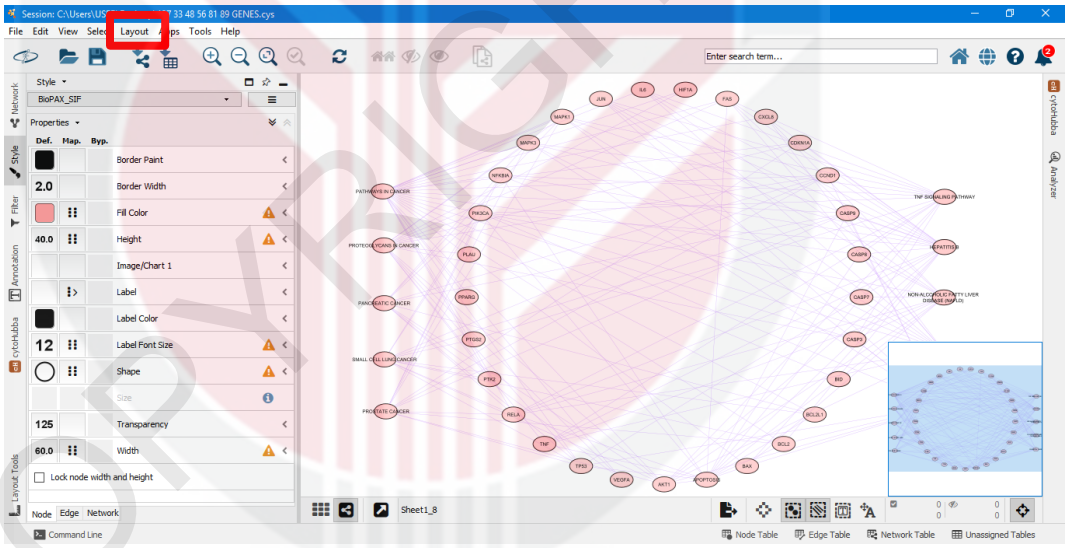
- The network will be shown like this after moving the pathway



- Arrange the node in a complete circle. Layout → attribute circle layout → selected nodes only → name



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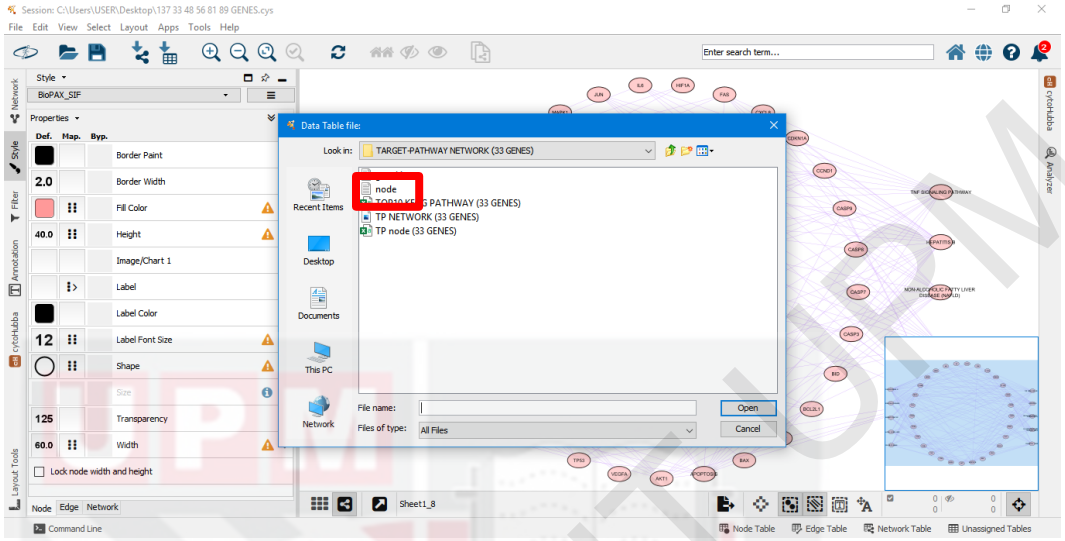
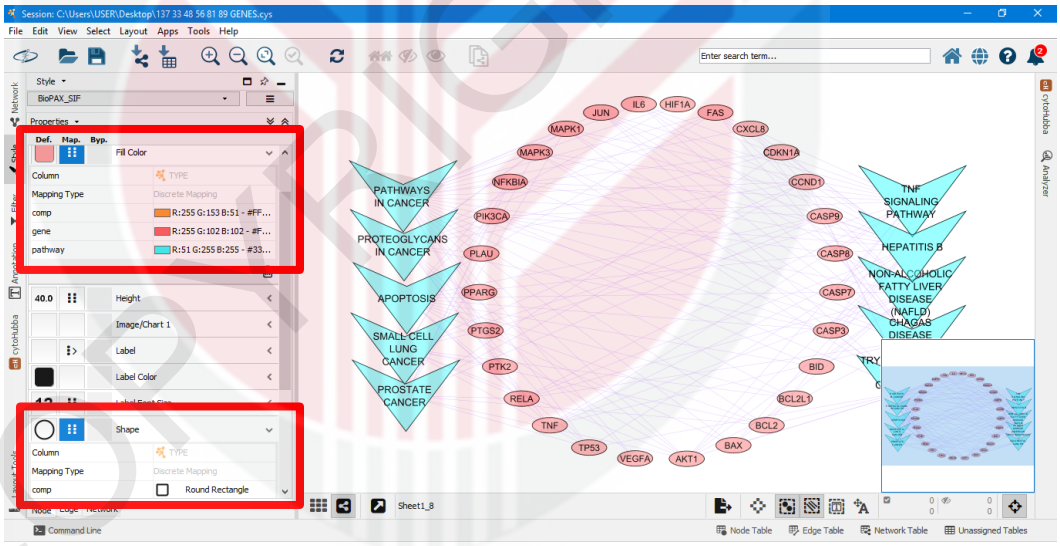
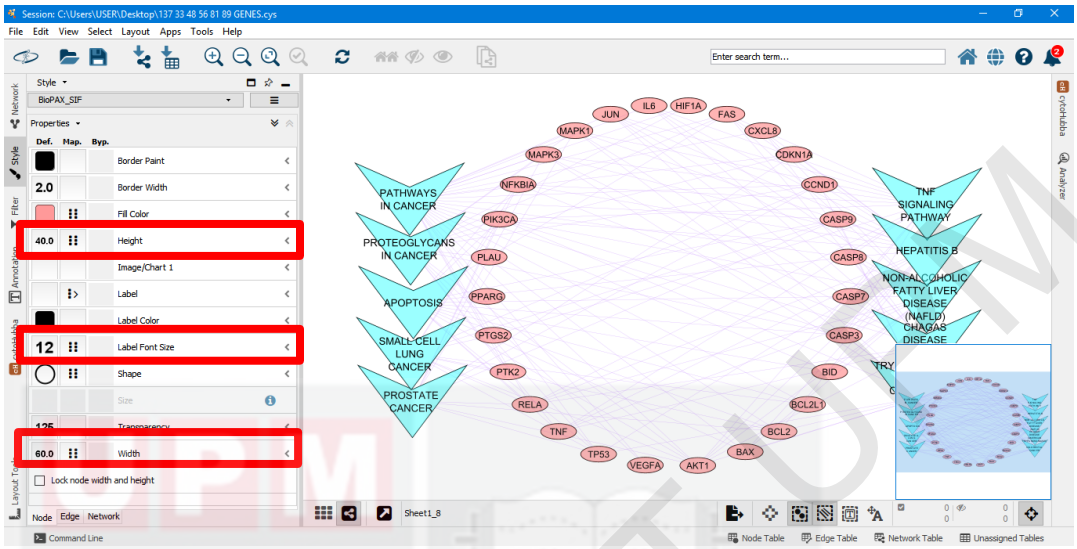
- To change the style of the node, import the node file to provide information for the Cytoscape. Import → Table from file → node



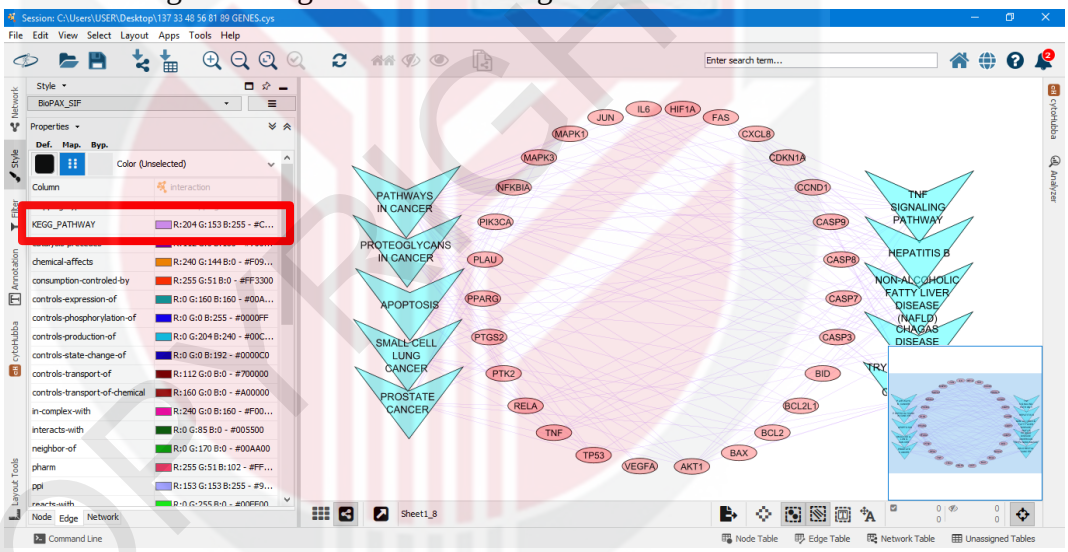
- Change the colour and shape of nodes of the pathway and gene to differentiate them



- The height and width of nodes as well as font size can also be changed



- Click Edge to change the colour of edges



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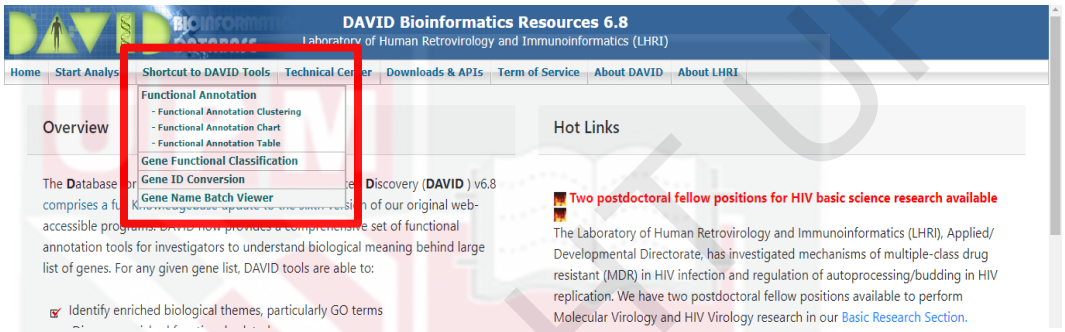
APPENDICES X

11.1 Gene ontology and pathway enrichment analysis

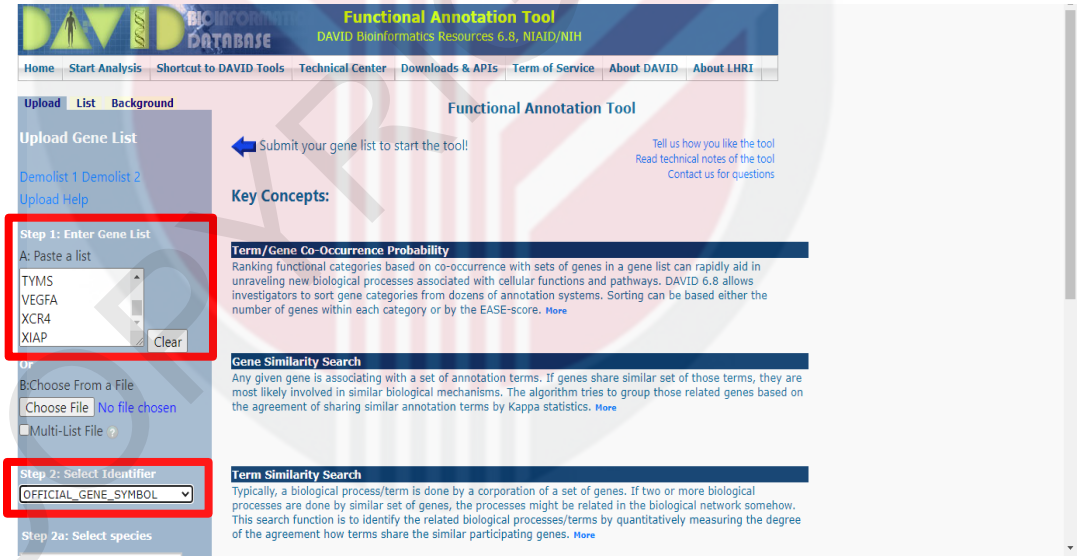
11.1

10.1.1 Gene enrichment analysis

- Go to the DAVID Bioinformatics Resources 6.8: https://david.ncifcrf.gov/summary.jsp. Click shortcut to DAVID tools → functional annotations



- Paste the overlapping genes → choose OFFICIAL_GENE_SYMBOL



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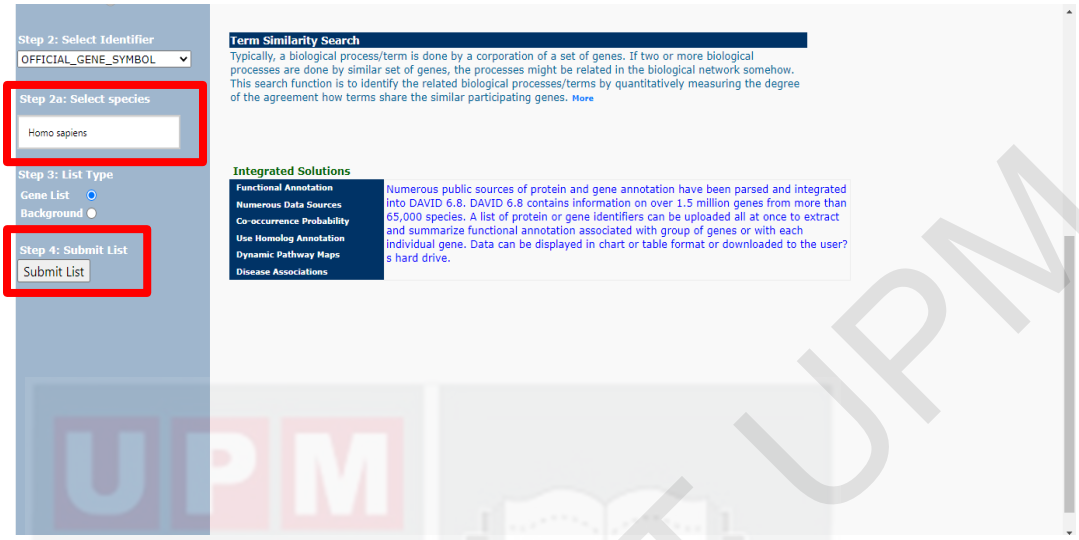
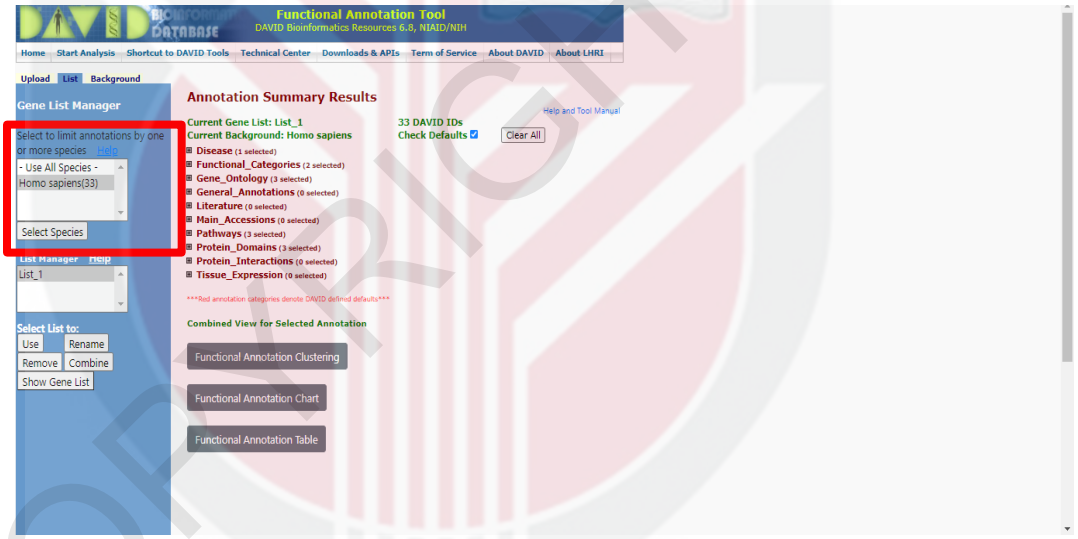
- Select Homo sapiens → gene list → submit



- The result will be shown. Select for Homo sapiens > select species > result



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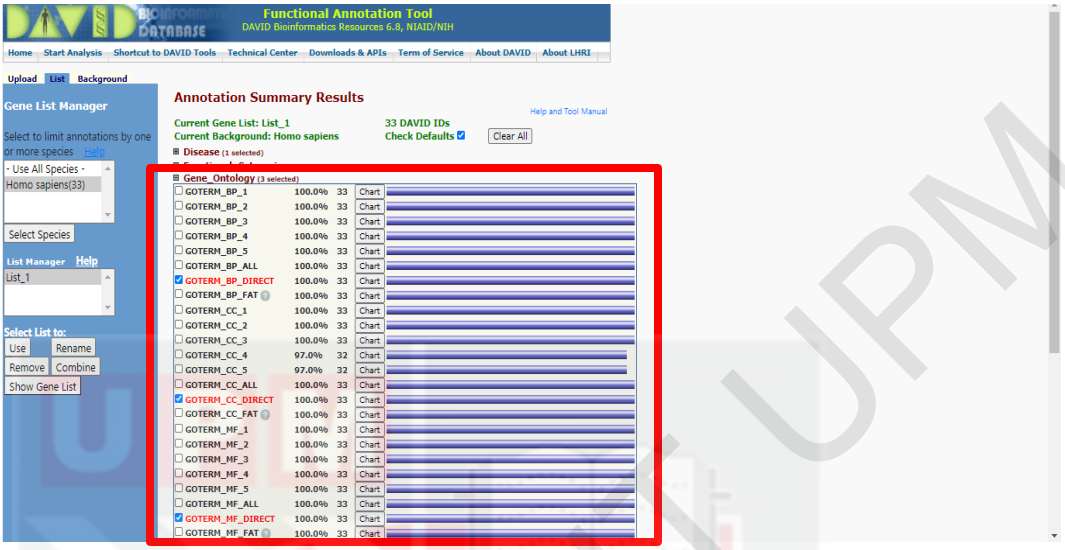
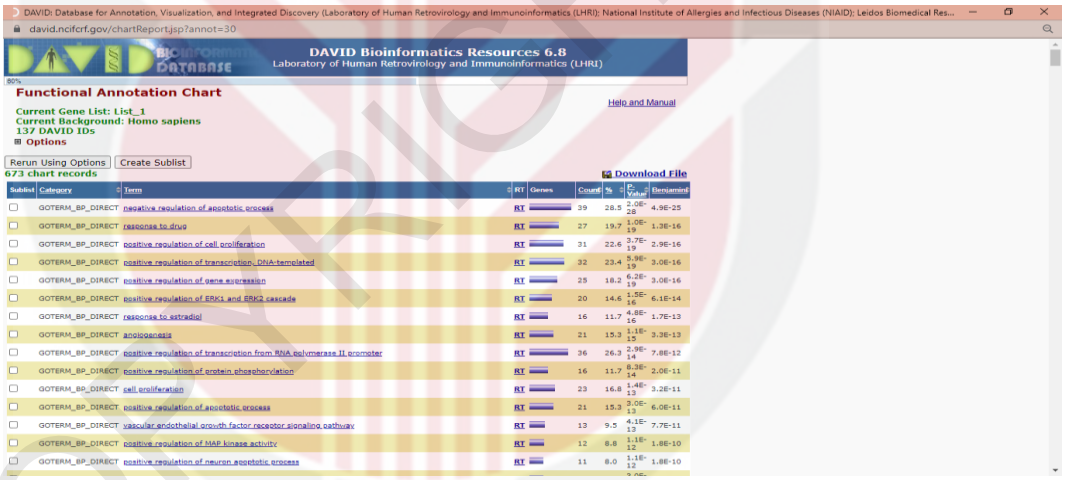
- Click for Gene_Ontology and chart for respective gene ontology terms to obtain the result



- The result will be shown and can be downloaded



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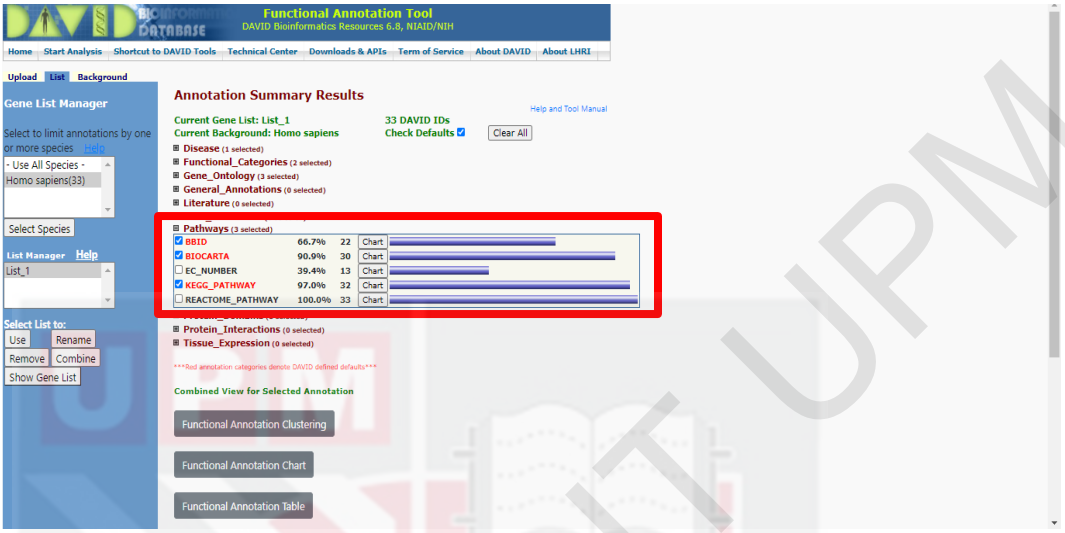
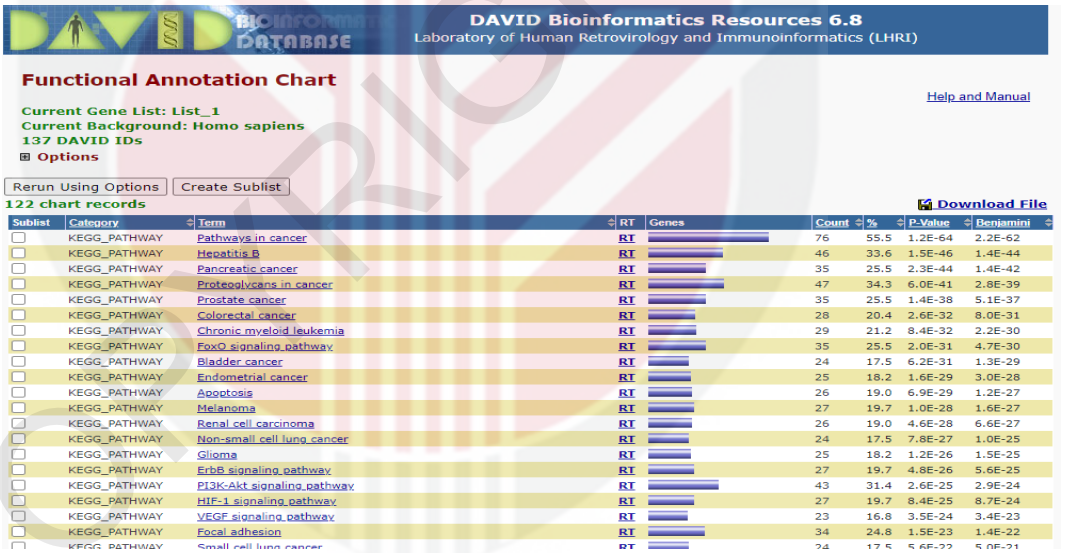
10.1.2 KEGG pathway enrichment analysis

- The similar step in performing gene ontology enrichment analysis. From the result of analysis, click pathway → chart for KEGG_PATHWAY



- The result will be shown and can be downloaded



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APPENDICES XI

11.1 The top 10 GO enrichment terms for MO+GEM-intersection against pancreatic cancer

Terms	ID	Biological Process	Gene count	P-value
Biological process	GO:0043066	Negative regulation of apoptotic process	39	2.04E-28
	GO:0042493	Response to drug	27	1.04E-19
	GO:0008284	Positive regulation of cell proliferation	31	3.67E-19
	GO:0045893	Positive regulation of transcription, DNA-templated	32	5.94E-19
	GO:0010628	Positive regulation of gene expression	25	6.21E-19
	GO:0070374	Positive regulation of ERK1 and ERK2 cascade	20	1.53E-16
	GO:0032355	Response to estradiol	16	4.82E-16
	GO:0001525	Angiogenesis	21	1.09E-15
	GO:0045944	Positive regulation of transcription from RNA polymerase II promoter	36	2.92E-14
	GO:0001934	Positive regulation of protein phosphorylation	16	8.35E-14
Cellular component	GO:0005829	Cytosol	75	8.32E-22
	GO:0005634	Nucleus	78	3.28E-11
	GO:0005737	Cytoplasm	76	4.93E-11
	GO:0005615	Extracellular space	34	6.32E-10
	GO:0005925	Focal adhesion	19	6.73E-10
	GO:0005654	Nucleoplasm	49	4.76E-09

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	GO:0043234	Protein complex	17	6.90E-08
	GO:0009986	Cell surface	19	1.05E-07
	GO:0005886	Plasma membrane	59	1.07E-07
	GO:0031093	Platelet alpha granule lumen	8	1.58E-07
Molecular function	GO:0005515	Protein binding	124	1.17E-23
	GO:0042802	Identical protein binding	39	9.42E-21
	GO:0019899	Enzyme binding	24	1.69E-15
	GO:0016301	Kinase activity	21	4.29E-15
	GO:0008134	Transcription factor binding	20	1.06E-12
	GO:0046982	Protein heterodimerization activity	22	1.10E-10
	GO:0019901	Protein kinase binding	20	1.40E-10
	GO:0008083	Growth factor activity	14	5.23E-10
	GO:0004713	Protein tyrosine kinase activity	13	6.45E-10
	GO:0019903	Protein phosphatase binding	10	1.57E-09

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APPENDICES XII

11.1 The top 10 GO enrichment terms for shared biotargets-intersection against pancreatic cancer

Terms	ID	Biological Process	Gene count	P-value
Biological process	GO:0043066	Negative regulation of apoptotic process	12	3.96E-10
	GO:0043065	Positive regulation of apoptotic process	10	3.22E-09
	GO:0042493	Response to drug	10	3.61E-09
	GO:0006974	Cellular response to DNA damage stimulus	9	3.95E-09
	GO:0097192	Extrinsic apoptotic signaling pathway in absence of ligand	6	4.85E-09
	GO:0006919	Activation of cysteine-type endopeptidase activity involved in apoptotic process	7	9.92E-09
	GO:2000811	Negative regulation of anoikis	5	2.54E-08
	GO:0042127	Regulation of cell proliferation	8	4.69E-08
	GO:0006915	Apoptotic process	11	5.87E-08
	GO:0001836	Release of cytochrome c from mitochondria	5	9.37E-08
Cellular component	GO:0005829	Cytosol	23	4.76E-10
	GO:0005634	Nucleus	24	1.02E-06
	GO:0005654	Nucleoplasm	17	4.36E-06
	GO:0005739	Mitochondrion	11	6.08E-05
	GO:0031264	Death-inducing signaling complex	3	6.24E-05

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	GO:0043234	Protein complex	7	7.09E-05
	GO:0005741	Mitochondrial outer membrane	5	1.29E-04
	GO:0005737	Cytoplasm	20	2.76E-04
	GO:0005667	Transcription factor complex	4	0.004627
	GO:0097136	Bcl-2 family protein complex	2	0.005259
Molecular function	GO:0042802	Identical protein binding	15	2.28E-11
	GO:0008134	Transcription factor binding	10	1.90E-09
	GO:0019899	Enzyme binding	10	7.64E-09
	GO:0005515	Protein binding	32	2.49E-08
	GO:0031625	Ubiquitin protein ligase binding	8	8.92E-07
	GO:0097153	Cysteine-type endopeptidase activity involved in apoptotic process	4	1.75E-06
	GO:0046982	Protein heterodimerization activity	9	1.83E-06
	GO:0019901	Protein kinase binding	8	5.35E-06
	GO:0051434	BH3 domain binding	3	2.08E-05
	GO:0032403	Protein complex binding	6	3.97E-05

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APPENDICES XIII

11.1 The top 10 GO enrichment terms for MO-intersection against pancreatic cancer

Terms	ID	Biological Process	Gene count	P-value
Biological process	GO:0043066	Negative regulation of apoptotic process	22	1.07E-15
	GO:0042493	Response to drug	16	8.16E-12
	GO:0001525	Angiogenesis	14	2.90E-11
	GO:0008630	Intrinsic apoptotic signaling pathway in response to DNA damage	9	4.08E-11
	GO:0048010	Vascular endothelial growth factor receptor signaling pathway	10	4.21E-11
	GO:0030168	Platelet activation	11	1.30E-10
	GO:0045944	Positive regulation of transcription from RNA polymerase II promoter	23	3.57E-10
	GO:0038095	Fc-epsilon receptor signaling pathway	12	5.85E-10
	GO:0043525	Positive regulation of neuron apoptotic process	8	9.99E-10
	GO:0033138	Positive regulation of peptidyl-serine phosphorylation	9	1.12E-09
Cellular component	GO:0005829	Cytosol	46	1.37E-14
	GO:0005634	Nucleus	47	1.25E-07
	GO:0005942	Phosphatidylinositol 3-kinase complex	5	3.17E-07
	GO:0005925	Focal adhesion	12	9.42E-07

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	GO:0005739	Mitochondrion	20	2.90E-06
	GO:0005654	Nucleoplasm	29	7.87E-06
	GO:0005911	Cell-cell junction	8	9.66E-06
	GO:0005737	Cytoplasm	42	1.13E-05
	GO:0005615	Extracellular space	19	1.39E-05
	GO:0043234	Protein complex	10	7.19E-05
Molecular function	GO:0042802	Identical protein binding	27	1.56E-16
	GO:0005515	Protein binding	73	1.15E-14
	GO:0019899	Enzyme binding	16	2.77E-11
	GO:0008134	Transcription factor binding	15	4.13E-11
	GO:0016301	Kinase activity	14	7.18E-11
	GO:0016303	1-phosphatudylinositol-3-kinase activity	7	4.26E-08
	GO:0019901	Protein kinase binding	13	1.45E-07
	GO:0042826	Histone deacetylase binding	8	4.44E-07
	GO:0046982	Protein heterodimerization activity	13	1.38E-06
	GO:0004674	Protein serine/threonine kinase activity	11	8.72E-06

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APPENDICES XIV

11.1 The top 10 GO enrichment terms for GEM-intersection against pancreatic cancer

Terms	ID	Biological Process	Gene count	P-value
Biological process	GO:0043066	Negative regulation of apoptotic process	29	6.29E-23
	GO:0008284	Positive regulation of cell proliferation	26	5.27E-19
	GO:0042493	Response to drug	21	5.76E-17
	GO:0045893	Positive regulation of transcription, DNA-templated	25	7.43E-17
	GO:0032355	Response to estradiol	14	1.03E-15
	GO:0043065	Positive regulation of apoptotic process	18	1.96E-13
	GO:0010628	Positive regulation of gene expression	17	3.44E-13
	GO:0008283	Cell proliferation	19	3.98E-13
	GO:0043406	Positive regulation of MAP kinase activity	11	4.18E-13
	GO:0006919	Activation of cysteine-type endopeptidase activity involved in apoptotic process	12	4.48E-13
Cellular component	GO:0005829	Cytosol	51	2.38E-16
	GO:0005737	Cytoplasm	54	3.53E-10
	GO:0005634	Nucleus	55	3.87E-10
	GO:0005654	Nucleoplasm	37	3.90E-09
	GO:0043234	Protein complex	14	7.50E-08
	GO:0009986	Cell surface	13	9.88E-06

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	GO:0005739	Mitochondrion	20	1.31E-05
	GO:0005901	Caveola	6	1.46E-05
	GO:0005925	Focal adhesion	11	1.69E-05
	GO:0005615	Extracellular space	19	5.53E-05
Molecular function	GO:0005515	Protein binding	82	5.59E-17
	GO:0042802	Identical protein binding	27	3.43E-15
	GO:0019899	Enzyme binding	18	9.78E-13
	GO:0046982	Protein heterodimerization activity	18	1.90E-10
	GO:0008134	Transcription factor binding	15	1.91E-10
	GO:0016301	Kinase activity	13	3.76E-09
	GO:0019901	Protein kinase binding	15	7.20E-09
	GO:0032403	Protein complex binding	11	1.04E-07
	GO:0019903	Protein phosphatase binding	7	8.45E-07
	GO:0042803	Protein homodimerization activity	17	8.52E-07

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APPENDICES XV

11.1 The KEGG pathway analysis associated with pancreatic cancer related target genes of MO and GEM

	ID	KEGG Pathway	Gene count	P-value
MO+GEM-intersection target genes against pancreatic cancer	hsa05200	Pathways in cancer	76	1.19E-64
	hsa05161	Hepatitis B	46	1.51E-46
	hsa05212	Pancreatic cancer	35	2.30E-44
	hsa05205	Proteoglycans in cancer	47	5.96E-41
	hsa05215	Prostate cancer	35	1.37E-38
	hsa05210	Colorectal cancer	28	2.57E-32
	hsa05220	Chronic myeloid leukemia	29	8.37E-32
	hsa04068	FoxO signaling pathway	35	2.03E-31
	hsa05219	Bladder cancer	24	6.15E-31
	hsa05213	Endometrial cancer	25	1.60E-29
Shared biotarget-intersection against pancreatic cancer	hsa05200	Pathways in cancer	25	1.33E-24
	hsa05161	Hepatitis B	19	3.71E-23
	hsa04210	Apoptosis	15	1.14E-21
	hsa04668	TNF signaling pathway	14	2.38E-16
	hsa05210	Colorectal cancer	11	6.35E-14

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	hsa05142	Chagas diseases (American trypanosomiasis)	12	3.63E-13
	hsa04932	Non-alcoholic fatty liver disease (NAFLD)	13	7.96E-13
	hsa05205	Proteoglycans in cancer	14	9.37E-13
	hsa05222	Small cell lung cancer	11	1.73E-12
	hsa05215	Prostate cancer	11	2.48E-12
	hsa05212	Pancreatic cancer	10	5.80E-12
MO-intersection target genes against pancreatic cancer	hsa05200	Pathways in cancer	49	3.46E-44
	hsa05161	Hepatitis B	33	2.83E-36
	hsa04210	Apoptosis	23	9.70E-30
	hsa05212	Pancreatic cancer	23	3.36E-29
	hsa05215	Prostate cancer	23	7.13E-26
	hsa05205	Proteoglycans in cancer	29	1.32E-25
	hsa05211	Renal cell carcinoma	20	1.18E-23
	hsa05210	Colorectal cancer	19	1.69E-22
	hsa04620	Toll-like receptor signaling pathway	22	2.26E-22
	hsa04668	TNF signaling pathway	22	2.79E-22
GEM-intersection target genes against pancreatic cancer	hsa05200	Pathways in cancer	52	1.89E-44
	hsa05161	Hepatitis B	32	2.31E-32

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hsa05205	Proteoglycans in cancer	32	9.701E-28
hsa05219	Bladder cancer	20	3.55E-27
hsa05212	Pancreatic cancer	22	5.70E-26
hsa05215	Prostate cancer	23	2.04E-24
hsa05210	Colorectal cancer	20	5.15E-23
hsa05213	Endometrial cancer	19	6.12E-23
hsa05220	Chronic myeloid leukemia	20	1.29E-21
hsa04068	FoxO signaling pathway	24	1.72E-21

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