UNIVERSITI PUTRA MALAYSIA

EVALUATION OF THE EFFECT OF LEMONGRASS AND GEMCITABINE COMBINATION TREATMENT ON PANCREATIC CANCER THROUGH NETWORK PHARMACOLOGY: FINDINGS OF BIOINFORMATICS ANALYSES

NORSYASYA ADRIANA BINTI ROSDI

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FPSK2 2021 16

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NORSYASYA ADRIANA BINTI ROSDI

A PROJECT PAPER SUBMITTED AS PARTIAL REQUIREMENT FOR THE DEGREE OF BACHELOR OF SCIENCE (BIOMEDICAL SCIENCES)

DEPARTMENT OF BIOMEDICAL SCIENCES
FACULTY OF MEDICINE AND HEALTH SCIENCES
UNIVERSITI PUTRA MALAYSIA

2021

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ABSTRACT

Evaluation of the Effect of Lemongrass and Gemcitabine Combination Treatment on Pancreatic Cancer through Network Pharmacology: Findings of Bioinformatics Analyses

Norsyasya Adriana Rosdi[a], Chenglong Xiong[b], Guozhong He[c], Karuppiah Thilakavathy[a,d]

[a]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[b]Department of Medical Microbiology, School of Public Health, Fudan University, Shanghai, 200032, People's Republic of China, China

[c]Institute of Health, Kunming Medical University, Kunming, 650500, China

[d]Genetics and Regenerative Medicine Research Group, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Introduction: Pancreatic cancer has contributed to a poor prognosis in cancer diagnosis as it is often detected at the more advanced stage. Gemcitabine (GEM) has replaced fluorouracil as the first-line chemotherapy drug for pancreatic cancer since 1997. However, the 5-year survival rate of a pancreatic cancer patient has not yet improved and GEM resistance is widespread. Lemongrass, commonly found in the region with lower incidence, was observed to have a therapeutic value for many kinds of cancers including pancreatic cancer. Therefore, it is assumed that lemongrass and GEM may effectively manage pancreatic cancer; however, their combined effect is still unclear. **Objective:** This study aims to evaluate the combined effect of lemongrass and GEM on pancreatic cancer through network pharmacology. **Methodology:** Lemongrass bioactive compounds were screened from Traditional Chinese Medicine Systems Pharmacology and Traditional Chinese Medicine Integrated Database. Potential gene targets of the compounds and GEM were predicted through Disease Gene Interaction database, DrugBank, Comparative Toxicogenomics Database, Swiss Target Prediction, and Chemmapper databases. Pancreatic cancer genes were retrieved from MalaCards and Online Mendelian Inheritance in Man databases. The intersection genes between lemongrass bioactive compound, GEM and pancreatic cancer were created using the online Venn Diagram tool. The STRING database and Cytoscape software were used to construct protein-protein interaction and compound-target-

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pathway network, respectively. The DAVID bioinformatics tool was utilized to conduct gene ontology (GO) and pathway analysis. **Results:** As a result, there were 27 compounds in lemongrass, however only luteolin (LUT) that met the drug screening requirements and were selected as the bioactive compound. LUT has a total of 296 potential gene targets, with 67 shared with pancreatic cancer while GEM has a total of 352 potential gene targets, with 89 shared with pancreatic cancer. Among those, several genes including *TP53, AKT1, VEGFA, EGFR, MYC, CCND1, STAT3, PTEN, CASP3, MAPK3,* and *BCL2L1* genes were identified as the hub genes with high biological relevance in pancreatic cancer. The GO and pathway analysis indicated that the combination treatment mainly acts on several cancer-related pathways, including pancreatic cancer, through apoptotic and cell proliferation regulation. **Discussion:** Hub genes are highly interconnected with nodes in the network and they were functionally enriched in multiple pathways. Therefore, it is believed that the potential mechanism is a synergy of multi-target and multi-pathway efforts. **Conclusion:** Since GEM treatment alone was commonly associated with the increased resistance development, this *in silico* study was conducted and has revealed that the effect of GEM in the pancreatic cancer treatment will be enhanced when in combination with LUT. Also, this study might provide an insight into lemongrass and GEM combination treatment against pancreatic cancer. However, further *in vitro* and *in vivo* experimental validation is needed to verify these computational findings.

Keywords: Lemongrass; Luteolin; Bioactive compound; Gemcitabine; Network pharmacology; Pancreatic cancer

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ABSTRAK

Kesan Penggabungan Serai dan Gemcitabine pada Kanser Pancreas melalui Farmakologi Rangkaian: Dapatan daripada Analisis Bioinformatik

Norsyasya Adriana Rosdi[a], Chenglong Xiong[b], Guozhong He[c], Karuppiah Thilakavathy[a,d]

[a]Jabatan Sains Bioperubatan, Fakulti Perubatan dan Sains Kesihatan, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

[b]Jabatan Mikrobiologi Perubatan, Sekolah Kesihatan Awam, Universiti Fudan, Shanghai, 200032, Republik Rakyat China, China

[c]Institut Kesihatan, Universiti Perubatan Kunming, Kunming, 650500, China

[d]Penyelidikan Perubatan Genetik dan Perubatan Regeneratif, Fakulti Perubatan dan Sains Kesihatan, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Pengenalan: Kanser pankreas menyumbang kepada prognosis buruk dalam diagnosis kanser kerana ia kebiasaannya dikesan pada tahap lebih teruk. Gemcitabine (GEM) menggantikan fluorouracil sebagai ubat standard piawaian kanser pankreas sejak 1997. Namun begitu, kadar kelangsungan hidup pesakit kanser pankreas selepas 5 tahun belum bertambah baik dan rintangan GEM adalah meluas. Serai, biasa dijumpai di kawasan yang rendah kejadian, mempunyai nilai terapeutik untuk pelbagai jenis kanser termasuk kanser pankreas. **Objektif:** Oleh itu, ia diandaikan serai dan GEM dapat mengurus kanser pankreas dengan berkesan; namun begitu, kesan penggabungan mereka masih tidak jelas. Kajian ini bertujuan untuk menilai kesan penggabungan serai dan GEM pada kanser pankreas melalui farmakologi rangkaian. **Metodologi:** Sebatian bioaktif serai ditapis daripada pangkalan data "Traditional Chinese Medicine Systems Pharmacology" dan "Traditional Chinese Medicine Integrated Database". Potensi gen sasaran sebatian dan GEM diramal melalui pangkalan data "Disease Gene Interaction database", "DrugBank", "Comparative Toxicogenomics Database", 'Swiss Target Prediction', dan "Chemmapper". Gen kanser pankreas diambil daripada pangkalan data "MalaCards" dan "Online Mendelian Inheritance in Man". Gen persilangan di antara sebatian bioaktif serai, GEM dan kanser pankreas dibentuk menggunakan alat gambar rajah Venn dalam talian. Pangkalan data STRING dan perisian Cytoscape masing masing digunakan untuk membina rangkaian interaksi protein-protein dan sebatian-sasaran-laluan. Alat

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bioinformatik seperti DAVID digunakan untuk mengendalikan analisis ontologi gen dan laluan. **Keputusan:** Hasilnya, terdapat 27 sebatian serai, namun begitu hanya luteolin (LUT) yang mencapai syarat saringan ubat dan dipilih sebagai sebatian bioaktif. LUT mempunyai sejumlah 296 potensi gen sasaran, dengan 67 dikongsi bersama kanser pankreas, manakala GEM mempunyai sejumlah 352 potensi gen sasaran, dengan 89 dikongsi bersama kanser pankreas. Di antara itu, beberapa gen termasuk *TP53, AKT1, VEGFA, EGFR, MYC, STAT3, CCND1, PTEN, CASP3, MAPK3,* dan *BCL2L1* dikenalpasti sebagai gen hub dengan perkaitan biologi yang tinggi dalam kanser pankreas. Analisis GO dan laluan menunjukkan rawatan gabungan kebanyakannya bertindak pada laluan yang berkaitan dengan beberapa dengan kanser, termasuk kanser pankreas, melalui pengawalan apoptosis dan percambahan sel. **Perbincangan:** Gen hub mempunyai perkaitan antara nod dalam rangkaian yang tinggi dan mereka berfungsi dalam pelbagai laluan. Oleh itu, ia dipercayai yang mekanisme berpotensi ialah usaha sinergi pelbagai sasaran dan pelbagai laluan. **Kesimpulan:** Memandangkan rawatan GEM sahaja sering dikaitkan dengan peningkatan pembentukan rintangan, kajian dalam silico ini dijalankan dan telah mendedahkan kesan GEM di dalam rawatan kanser pankreas akan dipertingkatkan apabila digabungkan dengan LUT. Kajian ini juga mungkin memberikan pandangan dalam rawatan gabungan serai dan GEM terhadap kanser pankreas. Namun begitu, pengesahan eksperimen dalam vitro dan vivo lebih lanjut diperlukan untuk mengesahkan dapatan pengkomputeran ini.

Kata kunci: Serai; Luteolin; Sebatian bioaktif; Gemcitabine; Farmakologi rangkaian; Kanser pankreas

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ACKNOWLEDGEMENTS

First and foremost, I thanked God the Almighty for all the blessing, courage, strength, and patience that He had given me. Those had helped me to go through challenges in completing my final year project since day one. A special gratitude to my supervisor, Dr. Thilakavathy Karuppiah for giving me the opportunity to do research that I am interested in and providing invaluable guidance throughout this project. My thesis journey would not have been smooth without her help and support. I am very thankful and indebted for her passion in sharing her knowledge and deeply inspired with her vision, motivation, and optimism in doing her work.

I also would like to thank my co-supervisors, Dr. Xiong Chenglong and Prof. He Guozhong for their guidance and response whenever I shared my research works. My sincere thanks also go to Dr. Sun Zhong for giving me the opportunity to conduct this project and also teaching and guiding me in this bioinformatics field.

I also take this opportunity to express my sincere appreciation to my final year project mates, Aina Nadheera and Nursaffa Alisya and all classmates for their endless support. I am incredibly grateful to have them and their positivity has inspired me to not give up with this project. Not to forget, I would always like to acknowledge my parents, Rosdi bin Hasan and Khatijah binti Jusoh and my siblings for their encouragement and understanding which had helped me to keep moving. Thank you very much.

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CHAPTER 1

INTRODUCTION

1.1 Background

Cancers arise when abnormal cells in any body organ or tissue grow and divide in an uncontrolled way, invade and spread to the other organs causing damage to the specific site. Not to mention, cancer is the most well-known disease worldwide as it is reported as a leading cause of death globally with almost 10 million deaths in 2020 (World Health Organization [WHO], 2021). Furthermore, the rate of new cases as well as death cases is growing fast worldwide (Sung et al., 2021). In Southeast Asia, cancer is classified as the top killers' disease. For example, in Malaysia, in 2016, cancer is the fourth leading cause of death as it contributes to 12.6% and 26.7% of all mortality cases in government hospitals and private hospitals respectively (National Cancer Registry, 2018).

Pancreatic cancer which develops within the tissue of pancreas is one of the cancers that has ranked as the 12th most common type of cancer worldwide (GLOBOCAN, 2020). Pancreas is an important organ located behind the stomach which is responsible in producing enzymes that aids in digestion and two hormones; glucagon and insulin that control glucose metabolism. At present, pancreatic cancer is regarded as the seventh leading cause of cancer death globally. Southeast Asia and Africa are among the regions with low incidence and mortality rates, however, because of its poor prognosis in cancer diagnosis, the mortality rates are as high as incidence rates (Sung et al., 2021). Also, pancreatic cancer showed a very lower relative

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survival rate compared to other types of cancer with 33.2% at 1-year and only 14.0% at 5-year (National Cancer Registry, 2018).

Till to date, surgery and chemotherapy are the most commonly used cancer therapies. Gemcitabine, a chemotherapeutic drug, acts as a first-line treatment for advanced pancreatic cancer (Saung & Zheng, 2017). However, the efficacy of the gemcitabine alone remains low with 20-25% of 5-year survival rates in 2005 (Vincent et al., 2007 & Boeck et al., 2011 as cited in Li et al., 2018) and resulted in lower level of survival outcomes when compared to gemcitabine-based combination therapies (Sun et al., 2012 & Ciliberto et al., 2013 as cited in Iyikesici, 2020). In a recent study, ginsenoside Rg3 was found to suppress the cell viability of GEM-resistant pancreatic cancer by inducing apoptosis (Zou et al., 2020). Another compound, geraniol extracted from *Cymbopogon citratus* enhanced the inhibitory effects of GEM on BXPC-3 human pancreatic cancer cells by inducing cell apoptosis (Jin et al., 2013). These findings prove that combination therapies could provide a better outcome when compared to monotherapy.

Cymbopogon citratus, commonly known as lemongrass, is an edible plant that has enormous striped leaves with uneven edges. They grow well in warm and moist environments like in numerous parts of the tropical and sub-tropical Southeast Asia and Africa (Wifek et al., 2016). It has been reported in many studies that lemongrass can treat different types of cancer such as lung, colon, prostate, breast, skin, liver, kidney and oral cancers (Tomko et al., 2020). In pancreatic cancer, perillyl alcohol, farnesol and geraniol which are the constituents of lemongrass essential oil have the ability to inhibit and

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suppress tumor growth (Burke et al., 1997). Another study also discovered that citral, geranial, neral and derivatives present in the lemongrass can induce cell death in pancreatic cancer patients by inhibiting the cell proliferation (Di Mola et al., 2017).

To predict lemongrass bioactive compounds and also study its effect in combination with gemcitabine against pancreatic cancer, network-based pharmacology would be one of the best approaches. In a recent study, network-based pharmacology was mentioned to be a promising approach for cost-effective drug discovery (Zhang et al., 2019). It is a rapidly developing computational approach as it is becoming an important mechanism to identify various compounds and therapeutic targets in traditional herbal medicine by amalgamating pharmacokinetic data and biological actions (Wang et al., 2013 as cited in Kim & Kim, 2020).

1.2 Problem Statement

Performing *in-vitro* and *in-vivo* pharmacology studies are generally expensive and more time consuming, therefore a preliminary *in-silico* study would be one of the best approaches that could improve this limitation. Up to now, Southeast Asian herbs are not well explored as a therapeutic agent for pancreatic cancer. Also, gemcitabine treatment alone against pancreatic cancer is commonly found to be associated with chemoresistance, and the combination of gemcitabine with herbal medicine like lemongrass is gaining much attention, however there is still lack of study on their combination in treating pancreatic cancer.

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1.3 Justification

Network pharmacology consisting of bioinformatics analysis is a cost-effective and time-saving approach which serve to theoretically predict the potential active compound of lemongrass and its effect in combination with gemcitabine in pancreatic cancer. Through literature review, lemongrass commonly found in Southeast Asia and Africa was observed to have a therapeutic value for many cancers including pancreatic cancer. Furthermore, to our knowledge, the study on the effect of lemongrass and gemcitabine combination treatment against pancreatic cancer has yet to be explored since it is a vital process to develop a drug which is able to effectively treat the disease.

1.4 Objectives

1.4.1 General Objective

This study generally aims to predict the target genes and the effect of lemongrass and gemcitabine combination treatment against pancreatic cancer, using bioinformatics approaches.

1.4.2 Specific Objective

To achieve the proposed general objective, the following specific objectives were

identified in this research:

I. To determine the potential bioactive compound associated with lemongrass using network pharmacology

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II. To screen for the potential pancreatic cancer-related targets of lemongrass and gemcitabine using online bioinformatic tools

III. To theoretically evaluate the effect of the combined lemongrass and gemcitabine treatment against pancreatic cancer by performing network analysis

1.5 Hypothesis

It can be hypothesized that lemongrass bioactive compound found from this study in combination with gemcitabine might have a synergistic effect in targeting the pancreatic cancer genes.

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CHAPTER 2

LITERATURE REVIEW

2.1 Pancreatic Cancer

2.1.1 Introduction

Pancreatic cancer is a gastrointestinal cancer which is recognised as one of leading causes of cancer death and one of the most lethal malignant neoplasms globally. The highest incidence and mortality rates of pancreatic cancer are commonly discovered in developed countries. The five-year survival rate for this cancer remains low compared to other types of cancer and this has remained constant throughout many years (Makohon-Moore et al., 2013). This type of cancer is derived from an organ located in the abdomen behind the stomach, known as pancreas.

Pancreatic cancer can be divided into two types which are exocrine pancreatic cancer and endocrine pancreatic tumours (Mitra, 2019). Exocrine pancreatic cancer or pancreatic adenocarcinoma are the most common types of pancreatic cancer which begins in the exocrine glands of pancreas with 85% occurrence. On the other hand, endocrine pancreatic tumours or neuroendocrine tumours are the least common types of pancreatic cancer which begins in endocrine tissue of pancreas with less than 5% occurrence. The symptoms usually appear at late stages which include abdominal pain, fatigue, jaundice, light-colored stools and weight loss (Rawla et al., 2019).

The causes of pancreatic cancer are still not fully understood, even though certain risk factors including cigarette smoking, obesity, diabetes mellitus, alcohol use, dietary factors and physical inactivity have been

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identified. Inherited risk factors also contributed to the development of pancreatic cancer with 5-10% approximately (Mizrahi et al., 2020).

2.1.2 Current Epidemiology

Pancreatic cancer is recently known as the 12th most common cancer worldwide and 7th leading cause of cancer mortality. Globally, 495,773 new cases and 466,003 deaths of pancreatic cancer have been reported for both genders (GLOBOCAN, 2020) and it is estimated that, in a short time, pancreatic cancer will surpass breast cancer as the third leading cause of cancer death (Mizrahi et al., 2020).

According to GLOBOCAN 2020, Southeast Asia reported a lower age-standardized incidence rate for pancreatic cancer with only 2.9 per 100 000 people compared with Eastern Europe with the highest age-standardized incidence rate of 9.9 per 100 000 people (Sung et al., 2021). In Malaysia, pancreatic cancer was not so common where the incidence rate ranked at 14th with a lower number of new cases according to GLOBOCAN 2020. National Cancer Registry (2018) has also reported a lower number of new cases of pancreatic cancer in Malaysia with only 1829 cases compared to other types of cancer with a higher number of new cases, for example, female breast cancer with 18206 cases and colorectal cancer with 13683 cases. However, the number of deaths reported is as high as the number of new cases may be because of its poor prognosis. In addition to poor prognosis, the survival rates of patients diagnosed with pancreatic cancer also affected by the lateness of disease detection. In Malaysia, pancreatic cancer showed a very lower relative survival rate compared to other types of cancer with 33.2% at one year of

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disease detection and only 14.0% at five year of disease detection (National Cancer Registry, 2018).

2.1.3 Gene Alteration and Molecular Pathology

Pancreatic cancer develops from normal to increasing grades of pancreatic intraepithelial neoplasia (PanIN), to invasive cancer. Similar as many other cancer, pancreatic cancer results from the accumulation of mutations. The multigenic nature of pancreatic cancer involved the alteration of three classes of genes which are oncogenes, tumour-suppressor genes, and genomic maintenance genes (Table 2.1). Previously, it was reported that there were more than 85% of pancreatic cancers have an activating point mutation in the *KRAS* gene while the inactivation of tumour-suppressor genes *CDKN2A*, *TP53*, *SMAD4/MADH4*, and *BRCA2* frequencies were reported to be 82%, 76%, 53%, and 10%, respectively (Li et al., 2004).

Table 2.1: Oncogenes, tumour-suppressor genes, and genomic maintenance genes commonly being altered in pancreatic cancer patients (Adapted from Li et al., 2004).

	Chromosomal location	Alteration frequency (%)
Oncogenes		
K-ras	12p	75–100
HER2/neu	17q	65–70
AKT2	19q	10–20
MYB	6q	10
Tumour-suppressor and genome-maintenance genes		
TP53	17p	40–75
*CDKN2A**	9p	27–98
CDKN2A†	9p	27–82
CDKN2B	9p	27–48
MADH4	18q	50–55
FHIT	3p	66–70
RBI	13q	0–10
BRCA2	13q	7–10
STK11	19q	5
MAP2K4	17p	4
ALK5	9q	1
TGFBR2‡	3p	1
TGFBR2§	3p	3
MLH1	3p	3

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KRAS gene is one of the most commonly mutated oncogenes found in most advanced stages of pancreatic cancers which are responsible in regulating the downstream of growth factor receptors signalling. There is also alteration involving tumour suppressor genes, such as *CDKN2A*, *TP53* and *SMAD4/MADH4* observed. The *CDKN2A* gene, which encodes the cell cycle regulator, is inactivated in more than 90% of pancreatic cancer patients. Other than that, mutation of the *TP53* gene was found to be strongly associated with cellular responses to cytotoxic stress by contributing to cell apoptosis and cell cycle arrest. Alteration of *SMAD4/MADH4* which encodes the transforming growth factor beta (TGFβ) signaling pathway has been observed in nearly 55% of pancreatic cancer patients. It is important to identify early mutations in *SMAD4/MADH4* since it was observed to be linked with a poorer prognosis and widespread metastases (Karanikas et al., 2016).

Mutational activation of the *KRAS* oncogene and telomere shortening is usually observed early in low-grade PanIN progression while inactivation of tumour suppressor genes happens in advanced PanIN and invasive carcinomas (Vincent et al., 2011). PanIN is the most common precursor lesion of infiltrating pancreatic ductal adenocarcinoma involving normal ductal epithelium of the pancreas. PanIN lesions progress from normal to PanIN-1 lesion as the result of an activating *KRAS* mutation. On the other hand, the development of inactivating mutations in *CDKN2A* is associated with the progression of PanIN-1 to PanIN-2 lesion while inactivating mutations of *TP53* and *SMAD4* occurred at the late stage and most commonly detected in PanIN-3 stage lesions (Makohon-Moore et al., 2013).

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2.1.4 Current Treatment

Over many years, treatment options will usually be made by the clinicians based on the staging of pancreatic cancer. For patients who are diagnosed with an early stage of pancreatic cancer and can undergo surgical resection, surgery is the only treatment of choice that provides curative potential (Kosmidis et al., 2016; McGuigan et al., 2018; Mizrahi et al., 2020). There is also evidence that the use of chemotherapy as an adjuvant treatment to surgery lead to the improvement of survival rates of pancreatic cancer patients (McGuigan et al., 2018). There is also a situation where clinicians offered the use of neoadjuvant chemotherapy to improve surgical margins in order for the patient to undergo surgery without problems (Kosmidis et al., 2016).

A pancreaticoduodenectomy, also known as Whipple procedure is the most common method of surgery where the tumours localised in the pancreatic head are removed. Duodenum, gallbladder, common bile duct, proximal jejunum, and a segment of the stomach were also removed during the procedure. Distal pancreatectomy, or total pancreatectomy can also be done for surgical management where distal pancreatectomy is for patients with tumours located in the body or tail of pancreas while total pancreatectomy is the procedure to remove the whole pancreas from the body (Mizrahi et al., 2020). However, it has been reported that patients who go through surgical management have a perioperative mortality of 4-18% and risk of complications after the surgery. Also, at the time of surgery, quite a high number of patients have locally invasive and micro-metastasis. Because of that, there is a higher probability of disease recurrence after operation. Therefore, adjuvant therapy

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is designed specifically to decrease the risk of disease recurrence (Polireddy & Chen, 2016).

For patients who receive a diagnosis of locally advanced and metastatic pancreatic cancer, medical management which is chemotherapy is the treatment of choice. This type of treatment requires the use of drugs such as gemcitabine and FOLFIRINOX (Mizrahi et al., 2020) to kill the cancer cell. Adjuvant chemotherapy and neoadjuvant chemotherapy are two types of chemotherapy.

Following operation, adjuvant chemotherapy has become compulsory to prevent early tumour recurrence (Klaiber et al., 2018). According to the previous study, the landmark randomised CONKO-001 study supports the use of adjuvant chemotherapy. This study compared the use of adjuvant gemcitabine after complete surgical resection with surgical management alone. The result showed a significantly improved median disease survival and overall five year survival of 20.7% vs 10.4% and ten year survival of 12.2% vs 7.7%.

On the other hand, the purposes of neoadjuvant chemotherapy are to eliminate micro-metastasis and shrink or reduce the size of the primary tumour. This will eventually reduce the incidence of tumour recurrence (McGuigan et al., 2018). It is important to include histological confirmation of pancreatic cancer upon neoadjuvant chemotherapy (Rossi et al., 2014). However, complications including biliary occlusion can still be developed in patients who receive neoadjuvant treatment where it delays surgery which then allow the cancer to progress to where it becomes unresectable (Oba et al., 2020).

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For patients who receive a diagnosed distant metastasis pancreatic cancer, systemic chemotherapy remains the primary treatment. The aim of systemic chemotherapy is to alleviate cancer-related symptoms and prolong the patient's life (Mizrahi et al., 2020).

2.2 Gemcitabine

2.2.1 Introduction

Gemcitabine is one of the chemotherapeutic drugs and gemcitabine (GEM)-based treatment is widely recognised as the standard first-line drug in treating patients with advanced pancreatic cancer (Zou et al., 2020). DNA synthesis inhibition is one of the most important mechanisms of action (MOA) of gemcitabine. There is also another additional MOA which are inhibition of enzyme; ribonucleotide reductase that are important to DNA and induction of apoptosis through caspase signalling (De Sousa Cavalcante & Monteiro, 2014). Gemcitabine can be administered alone as mono-chemotherapy. However, the effect in treating advanced pancreatic cancer may not be promising compared to gemcitabine-based combination therapy.

National Comprehensive Cancer Network (NCCN) guidelines have mentioned that the therapeutic effect of gemcitabine improved in the gemcitabine-based combination therapy. Furthermore, in the first half year, the progression free survival in advanced pancreatic cancer patients treated with gemcitabine-based combination therapy was notably higher compared to treated with gemcitabine alone. However, drug combination therapy is often associated with undesirable effects (Jin et al., 2017). This will alter the treatment efficiency leading to chemotherapy failure.

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In addition to undesirable effects, gemcitabine also develops resistance in the clinical setting (De Sousa Cavalcante & Monteiro, 2014). Therefore, it is urgently needed to develop anti-cancer agents against side effects and gemcitabine chemoresistance by combining the existing modern medicine with traditional medicine.

2.2.2 Combination Based-Therapy with Traditional Herbal Medicine

In a recent study ginsenoside Rg3, isolated from *Panax ginseng*, was used by the researchers to determine the efficacy of those compounds against gemcitabine-resistant pancreatic cancer cells. Two types of cells which are Panc-1/GEM and SW1990/GEM cells were treated with different ginsenoside Rg3 concentrations for 48 hours and colony formation were examined. The result showed that the colony formation of two treated cells were significantly inhibited by ginsenoside Rg3. These prove that ginsenoside Rg3 could exhibit the anti-cancer effect in gemcitabine-resistant pancreatic cancer cells (Zou et al., 2020).

Other than that, baicalein (BAI) which is a purified flavonoid isolated from *Scutellaria baicalensis* root was found to enhance the effects of gemcitabine treatment in inhibiting the human CFPAC-1 pancreatic ductal adenocarcinoma cell viability *in vitro* via apoptotic process (Li et al., 2018).

Furthermore, in s previous study, the researchers investigated the inhibitory effect of geraniol alone, or in combination with gemcitabine on the proliferation of BXPC-3 human pancreatic cancer cells. Geraniol is a natural acyclic monoterpene compound extracted from different types of plants including P*elargonium, Cymbopogon spreng, Lauraceae Litsea* and *Rosaceae*.

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At the end of the study, the result demonstrated that geraniol enhanced the inhibitory effects of gemcitabine on BXPC-3 human pancreatic cancer cells by inducing cell apoptosis.

Therefore, it was believed that these findings on the gemcitabine combination-based therapy can be useful in clinical application that could provide more promising treatment for pancreatic cancer compared to existing treatment options (Jin et al., 2013).

2.3 Lemongrass

2.3.1 Introduction

Lemongrass, which is commonly known for its lemony and herbaceous fragrance is an edible grass plant with enormous striped green leaves (Wifek et al., 2016). It has the ability to grow up to 6 inch high. As for the leaf, the height is around 100 cm in length and 2 cm in width. Several studies have reported that lemongrass originated from Asia (Malaysia, Indonesia, Indochina), Africa and the Americas (Olorunnisola et al., 2014). Moreover, it is believed that the lemongrass name is actually derived from the distinctive lemon-like odour present in the shoot of the plant (Skaria et al., 2006). *Cymbopogon citratu*s, one of the lemongrass species belong to the genus *Cymbopogon* is widely used in Southeast Asia as spices as it gives flavour to the foods and also, one of the main sources of essential oils used for medicinal reasons (Trang et al., 2020).

2.3.2 Traditional Uses

Lemongrass has long been used in traditional medicine to treat several ailments even though the scientific data on medical claims is limited. In

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different parts of the world, lemongrass was used as the treatment for fever, digestive and menstrual disorders, rheumatism and other joint pains. Previously, it has been reported that the infusion forms of lemongrass can treat digestive and nervous disorder, inflammation, and also fever (Nambiar & Matela, 2012). In Ayurveda, lemongrass was used as a diuretic, tranquilizer, anti-inflammatory and antipyretic medicine. There are also several examples of lemongrass usage as health remedies by different ethnic groups. For instance, In Cuba, it is used to lower the blood pressure and treat rheumatism and mucus build-up in an airway or cavity of the body, while in Argentina, it is used to cure the sore throat and indigestion. In Brazil, tea prepared from lemongrass leaves is primarily used as an analgesic, sedative, diuretic, antispasmodic, antipyretic, and anti-inflammatory compound (Haque et al., 2018). Other than Brazil, tea made from lemongrass leave is also popular among countries of West Africa, Asia and South America. It has been broadly utilized as antifever, antiseptic, carminative, anti-dyspeptic, and anti-inflammatory effects (Olorunnisola et al., 2014). Gastrointestinal (GI) disturbances usually present with the symptoms of heartburn, stomach pain, constipation, diarrhea, nausea, and vomiting also has been reported to be traditionally treated with lemongrass (Trang et al., 2020).

2.3.3 Phytochemical Properties

The therapeutic value of medicinal plants might possibly be associated with the presence of its phytochemical constituents or also known as secondary metabolites. According to a previous study, essential oil (EO) and flavonoids are the most essential compounds in lemongrass, which contributed to the therapeutic and pharmacological activities of the plant (Oladeji et al., 2019).

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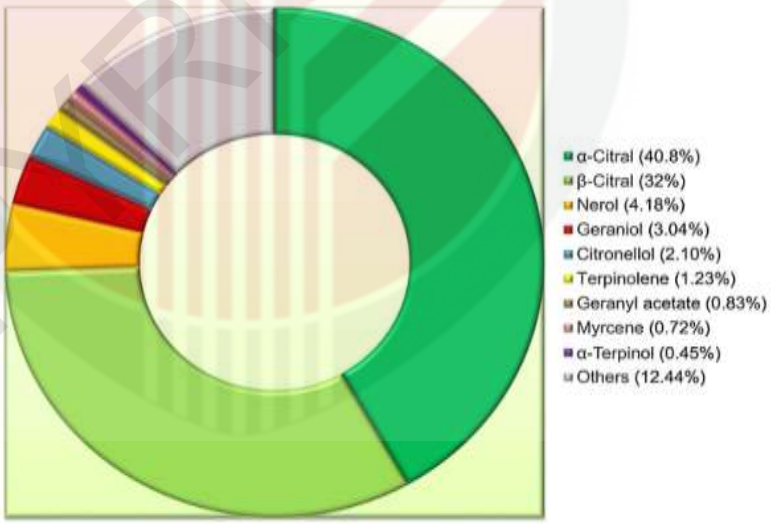
It has been reported that lemongrass mainly contains EO with citral contributed to the high composition (70–80%) of the compounds. This finding makes citral regarded as the main component of lemongrass EO. Citral is essential in providing lemon-like flavour to the plants (Oladeji et al., 2019) and made up of two stereo-isomeric monoterpene aldehydes: trans-citral and cis-citral, geranial and neral. It is generally found greater than 45% in lemongrass EO, however, the amount can be varied among species. As shown in Figure 2.1(a), other compounds including nerol, geraniol, citronellol, terpinolene, geranyl acetate, myrcene, and α-terpinol were also isolated from lemongrass EO with different composition (Haque et al., 2018; Nambiar & Matela, 2012). The chemical structure of the major phytochemical constituents in lemongrass EO were presented in Figure 2.1(b).

(a)



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(b)

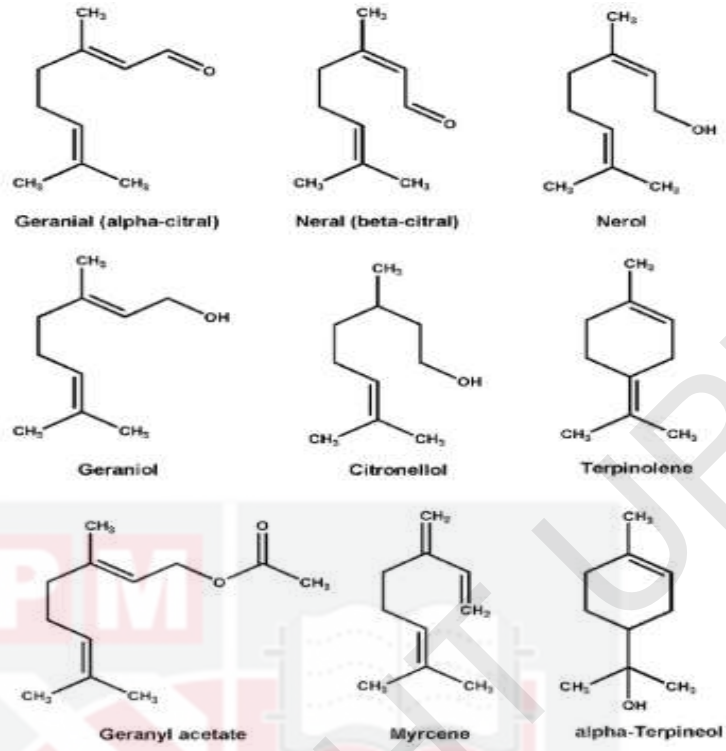

Figure 2.1: Major phytochemical constituents in lemongrass essential oil. The figure showed **(a)** the percent compositions and **(b)** chemical structures of compounds (Adapted from Haque et al., 2018).

Furthermore, flavonoids and phenolic compounds such as luteolin, apigenin, 7-O-glycosides, kaempferol, caffeic acid, catechol, elemicin and hydroquinone are another class of compounds identified in lemongrass (Oladeji et al., 2019). Flavonoids, the largest group of naturally occurring polyphenolic compounds contain two benzene rings separated by a propane unit (Figure 2.2(a)) with a subclass of flavones and flavanols becoming the most widely distributed of all the phenolics. On the other hand, phenolic compounds, the largest group of phytochemicals accounting for most of the antioxidant activity in plants contain an aromatic ring with one or more hydroxyl groups (Figure 2.2(b)) (Sulaiman & Balachandran, 2012).

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(a) **(b)**

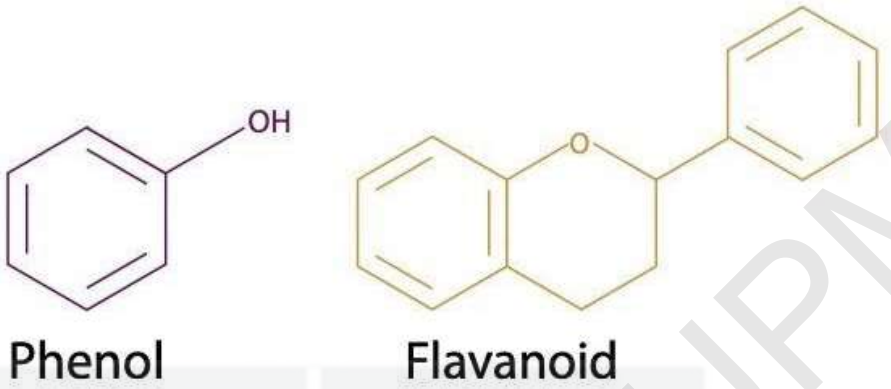

Figure 2.2: The chemical structures of phenolic compounds. **(a)** Flavonoids consist of two benzene rings separated by a propane unit. **(b)** Phenols contain only one benzene ring with one hydroxyl group or can be more (Adapted from Angelosante, 2020).

Lemongrass was also reported to contain another class of compounds which are terpenoids and mineral constituents. Several important terpenoids such as cymbopogone and cymbopogonol were isolated from lemongrass leaves. As for mineral content, potassium, magnesium, sodium, iron, manganese, zinc, and phosphorus have been identified (Oladeji et al., 2019).

2.3.4 Pharmacological Activities

Many studies have demonstrated that lemongrass potentially has numerous pharmacological activities. These includes anti-microbial, anti-inflammatory, anti-oxidant, and anti-cancer activity.

The antimicrobial activity of lemongrass has been investigated in many studies. For instance, the ethanolic extracts of the lemongrass leaves containing flavonoids and tannins exhibited potential antibacterial action against *Staphylococcus aureus* (Qadir & Fatima, 2017). Another study *in vitro*

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demonstrated that lemongrass EO elicits anti-bacterial activity against *Streptococcus pyogenes*. This species was found to play an important role in the development of tonsillitis (Battaglia, 2019). Moreover, two compounds of lemongrass EO which are geranial and neral were found to exert its antibacterial action on gram-negative and gram-positive organisms individually (Shah et al., 2011).

As for anti-inflammatory activity, an *in vivo* study has revealed that lemongrass essential oil and its flavonoids and tannins have the ability to reduce rat paw edema induced by carrageenan by inhibiting the synthesis and release of inflammatory mediators (Kassahun et al., 2020). Citral, luteolin, epoxyestragole, carvone, 6,7-epoxycitral, peritoneal and 6,7-epoxycitronellal, glycoside (O-,C-) and 8,9-epoxycarvone isolated from lemongrass also inhibits the secretion of prostaglandins (PGE2), an inflammatory mediator and nitric oxide (NO) associated with inflammation (Oladeji et al., 2019). Other than that, luteolin and its glycosides extracted from lemongrass exert its anti-inflammatory action by inhibiting nitric oxide (NO) and interleukin-1b (Wang et al., 2017).

Methanol/water extracts, infusion and decoction of lemongrass were manifested to have free radical scavenging effects in human erythrocytes by scavenging the superoxide anion and inhibiting the xanthine oxidase enzyme and lipoperoxidation (Shah et al., 2011; Olorunnisola et al., 2014). Another class of compounds found in lemongrass which are phenolic acid and flavonoids could also act as natural antioxidants and free radical scavengers (Qadir & Fatima, 2017).

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The anti-cancer activity of lemongrass has also been studied. Its EO may provide a defense mechanism against various kinds of tumours. For example, an *in vivo* study showed that administration of lemongrass EO could inhibit cancer tumours through the apoptotic process. Lemongrass was also found to stop cervical cancer cells from proliferating, and also able to initiate cancer cell apoptosis (Kassahun et al., 2020). In another study, lemongrass has the ability to both reduce growth of colon cancer xenografts and prevent the cancer formation in transgenic mice susceptible to developing colon cancer (Ruvinov et al., 2019). Moreover, luteolin, a flavonoid, has been proven to manage pancreatic cancer by induction of apoptotic process, cell cycle arrest, and suppression of the protein phosphorylation and signalling (Imran et al., 2019). Previous study in orthotopic mouse models have demonstrated that the combination of luteolin with gemcitabine could prevent the growth of pancreatic cancer. This combination induced programmed cell death in pancreatic cancer cells by inhibiting the K-ras/GSK-3β/ NF-κB signaling pathway and eventually reducing the bcl-2/bax ratio allowing for cytochrome c to release from the mitochondria and activating caspase 3 (Johnson et al., 2015).

2.4 Bioinformatic Approaches

2.4.1 Introduction

Bioinformatics is a huge and complex tool of computational technology. It involved multi- or interdisciplinary fields of science where many tools have been developed over time to constantly analyse massive amounts of data (Goujon et al., 2010). In recent studies on ovarian cancer, the use of bioinformatic approaches including PPI network analysis, module and

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survival analysis as well as miRNA-hub gene network construction have been demonstrated to be effective and reliable. This means integrating bioinformatics analysis might be helpful in discovering the biomarkers and the mechanisms underlying the cancer formation and progression (Wang et al., 2020). There is also a study mentioned the use of bioinformatic analysis to identify the key genes and related pathways in lung cancer (Y. Wang et al., 2021).

2.4.2 Network Pharmacology

Network pharmacology is a newly developed computational approach which targets all the important genes in such a complex condition of disease. It is very useful in the study of complex disease, for instance, cancer, where the disease state is mainly due to the mutation of multiple genes not a single gene (Muhammad et al., 2018). Previously, the father of Network Pharmacology named Hopkin, stated that multiple nodes in the network of disease can be targeted by a single drug (Hopkins, 2008). Moreover, for the evaluations of network pharmacology, there were three major steps involved which are prediction of bioactive targets, enrichment analysis of involved targets, and network construction between bioactive compounds, targets, and pathways as well as its analysis (Figure 2.3). According to recent study, to conduct the network pharmacology evaluation, there were variety of online databases available which include traditional Chinese medicine (TCM) databases, compound and drug information databases (Drugbank), STITCH, PubChem, target interaction databases (STRING) and gene-disease association databases (OMIM) (Zhang et al., 2019).

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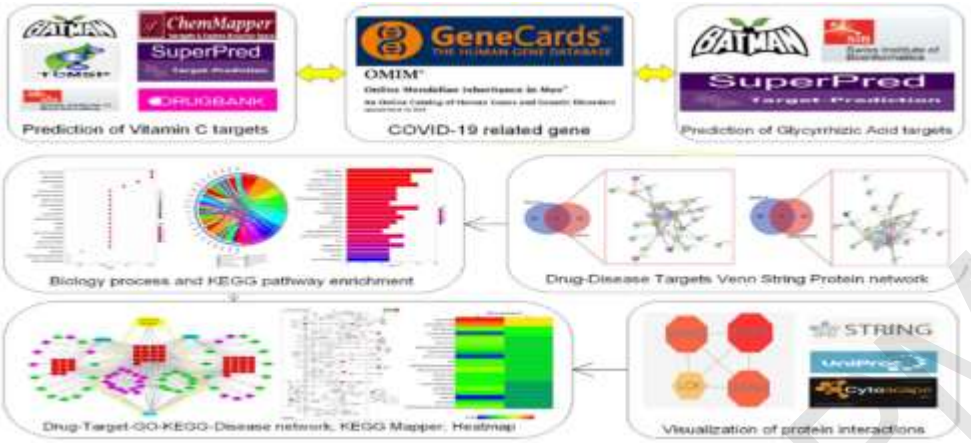

Figure 2.3: An integration approach of network pharmacology. This figure shows a stepwise workflow of the combined antiviral activity of VC and GA against COVID-19 through network pharmacology (Adapted from Li et al., 2020).

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CHAPTER 3

METHODOLOGY

3.1 Identification of the Potential Bioactive Compounds in Lemongrass

The potential bioactive compounds in lemongrass were retrieved from Traditional Chinese Medicine (TCM) databases, such as, Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (https://old.tcmsp-e.com/tcmsp.php) (Lee & Oh, 2020) and Traditional Chinese Medicine Integrated Database (TCMID) (http://119.3.41.228:8000/tcmid/) (Xu et al., 2020). In the TCMSP database, "Herb name" option was selected and the keyword "Xiang Mao" was entered. The molecule name of the compound contained in lemongrass was then displayed. In the TCMID website, "Herb" option was selected, the keyword "Lemongrass" was entered and the ingredient name contained in lemongrass were then listed. Refer Appendix I to see the steps to retrieve the potential bioactive compounds in lemongrass from TCMSP and TCMID.

3.2 *In-silico* Screening of Bioactive Compound of Lemongrass

All of the potential bioactive compounds of lemongrass undergo Absorption, distribution, metabolism and excretion (ADME) screening in order to be selected as the potential active compounds of lemongrass. In this study, the chemical compounds in lemongrass will be screened based on three most widely used parameters which are oral bioavailability (OB), drug- likeness (DL) and Caco-2 permeability. Oral bioavailability (OB) refers to the amount and rate of drug absorbed into the bloodstream after being administered orally (Chi et al., 2019). A high oral bioavailability minimizes the amount of an

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administered drug in order to achieve a desired effect while a poor oral bioavailability reduces the drug efficacy and therefore can lead to unpredictable response to a drug (Proietti et al., 2014). Next is drug-likeness (DL). Drug-likeness refers to the similarity between the compound and all known drugs available in the database. The accuracy of traditional Chinese medicine (TCM) compound screening will be improved by evaluating the drug-likeness of the compound (Chi et al., 2019). The last parameter is Caco-2 permeability which determines the drug absorption in the intestine. The human intestinal cell line Caco-2 is commonly used to study the drug absorption in the intestinal epithelium (Lu et al., 2020). All of the parameters were evaluated through TCMSP database. Refer to Appendix II to see the value of OB, DL and Caco-2 permeability for each compound. The compounds that meet the criteria of OB $\geq 30\%$, DL ≥ 0.18 and Caco-2 permeability ≥ -0.4 were considered as the bioactive compound of lemongrass and those that do not meet the criteria were excluded from the screening process (Lee & Oh, 2020).

3.3 Target Identification

3.3.1 Target Genes of Lemongrass Bioactive Compound

The PubChem database was first chosen to be the main source of chemical structure information for the active compound of lemongrass which includes canonical SMILES, molecular structures and standard database format (SDF) files (Xu et al., 2020). Then, by using those information, the target genes of lemongrass active compound were retrieved from the Drug Gene Interaction database (DGIdb) (https://www.dgidb.org/), Comparative Toxicogenomics Database (CTD) (http://ctdbase.org/) (Sun et al., 2020), SwissTargetPrediction database (http://www.swisstargetprediction.ch/) and ChemMapper database

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(http://www.lilab-ecust.cn/chemmapper/) (Li et al., 2020). All of the genes selected were limited to "Homo Sapiens". Genes that do not belong to "Homo Sapiens" were excluded from the list. Also, all of the target genes collected from different databases were undergoing cross-checking and the duplicated target genes were removed from the list. Refer to Appendix III to see the steps to retrieve the target genes of lemongrass bioactive compounds from online databases mentioned previously.

3.3.2 Target Genes of Gemcitabine

The target genes of gemcitabine were retrieved from the Drug Gene Interaction database (DGIdb) (https://www.dgidb.org/), Drugbank database (https://go.drugbank.com/) (Lee & Oh, 2020) and Comparative Toxicogenomics Database (CTD) (http://ctdbase.org/) (Sun et al., 2020). Only genes that belong to "Homo Sapiens" were included in the list while those do not belong to "Homo Sapiens" were excluded. After eliminating duplicates, the target genes of gemcitabine were finally shortlisted. Refer to Appendix III to see the detailed steps to retrieve the target genes.

3.3.3 Target Genes of Pancreatic Cancer

The target genes of pancreatic cancer were collected from two databases, namely, MalaCards: The human disease database (https://www.malacards.org/), which is an online database that provide information on human disease (Zhou et al., 2020) and Online Mendelian Inheritance in Man (OMIM) (https://www.omim.org/), which is an online database as well that continuously updating human genes and genetic disease. The keyword 'pancreatic cancer' was used in the Malacards and OMIM

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databases to search for pancreatic cancer-related targets (Meng et al., 2019). Refer to Appendix III to see the detailed steps to retrieve the target genes.

3.4 Screening Potential Targets of Lemongrass and Gemcitabine

Intersection analysis using an online tool (http://bioinformatics.psb.ugent.be/webtools/Venn/) were performed for all shortlisted target genes of lemongrass, gemcitabine and pancreatic cancer. Refer to Appendix IV to see how to perform the intersection analysis. As a result, the Venn diagram was created to show correlative target genes of lemongrass and gemcitabine against pancreatic cancer (Li et al., 2020) and also to screen for pancreatic cancer-related target genes of lemongrass and gemcitabine. The intersectional target genes were considered as the potential therapeutic targets of lemongrass and gemcitabine in pancreatic cancer.

3.5 Protein-Protein Interaction Network

The pancreatic cancer-related target genes of lemongrass and gemcitabine as well as the combination of the two were inputted into "Multiple Proteins" in the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org/, version: 11.0) to construct protein-protein interaction (PPI) network (Huang et al., 2020). The organism was limited to "Homo sapiens", the minimum required interaction score was set to "highest confidence" (> 0.9) and hide disconnected nodes in the network was chosen in the network display options. Refer to Appendix V to see how to construct a PPI network using STRING.

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3.6 Identification of Hub Genes

The PPI network with default settings were imported into Cytoscape software, version 3.8.2 (https://cytoscape.org/) for the identification of hub genes. The filtration of top 10 genes was conducted through Cytoscape plug-in cytoHubba based on three commonly used parameters which are "Degree", "Closeness" and "Betweenness" to calculate the topological features of each node in the network. Refer to Appendix VI to see the detailed steps to identify hub genes. Degree can be defined as the number of edges linked to a node and it reflects connectivity within the network, closeness is the shortest paths from node to other nodes and betweenness is the number of shortest paths passing through a node. By taking the intersection between those three parameters using the Venn diagram tool, hub genes were finally obtained.

3.7 Network Construction

The PPI network with default settings were further used for network construction. Network construction was performed using Cytoscape software, version 3.8.2 (https://cytoscape.org/), an open-source visualization software to display the compound-target-pathway interaction between lemongrass-gemcitabine and pancreatic cancer. There were two types of networks constructed which are compound-target (C-T) network and target–pathway (T-P) network (Jian et al., 2020). The C-T network was constructed by linking the lemongrass bioactive compounds and gemcitabine as well as the combination of the two with the corresponding target gene. On the other hand, the T-P network was constructed by linking the pancreatic cancer-related target genes of lemongrass bioactive compounds and gemcitabine as well as the

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combination of the two with the pathways involved. Refer to Appendix VII to see the detailed steps to construct C-T and T-P network using Cytoscape software. These networks are made up of nodes and edges where the nodes usually represent the compounds, genes, or pathways while the edges represent the interaction between them (Guo et al., 2019). Every node in the interaction network was analysed and nodes with higher ranks were considered to play an important role within the network (Wu et al., 2020).

3.8 Gene Ontology and Pathway Enrichment Analysis

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was conducted using the functional annotation tool of DAVID Bioinformatics Resources 6.8 (https://david.ncifcrf.gov/) by entering the list of target gene names and selecting the species as "Homo sapiens" (Guo et al., 2019). All target genes were named to their official gene symbol. Terms with thresholds of Count $\geq$ 2 and Expression Analysis Systematic Explorer (EASE) scores $\leq$ 0.5 were set according to default settings. These enrichment analyses were performed to reveal the underlying mechanism of combination lemongrass and gemcitabine treatment against pancreatic cancer through biological processes, molecular function, cellular components and key signalling pathways (Xia et al., 2020). Refer to Appendix VIII to see the detail steps to conduct the enrichment analysis using DAVID.

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CHAPTER 4

RESULTS

4.1 Screening and Identification of Bioactive Compounds in Lemongrass

A total of 27 candidate compounds of lemongrass were collected from Traditional Chinese Medicine Systems Pharmacology (TCMSP) database and Traditional Chinese Medicine Integrated Database (TCMID) (Table 4.1). Among those, 10 compounds including luteolin, (R)-linalool, l-limonene, beta-citronellol, 3-Methyl-3-hepten-2-one, p-coumaric acid, (-)-Citronellal, EEE, OXA and (2S)-2-methylbutan-1-ol were found to passed one of the drug screening criteria which is OB ≥ 30% and only one compound which is luteolin that met the other two drug screening criteria of DL ≥ 0.18 and Caco-2 permeability ≥ −0.4. It is concluded that luteolin was the only candidate compound that met the requirement of three drug screening criteria with the value of 36.16%, 0.25 and 0.19 for OB, DL and Caco-2 permeability, respectively. Therefore, this compound was selected as the bioactive compound of lemongrass and was further used to conduct the analyses.

Table 4.1: The ADME properties of 27 candidate compounds of lemongrass from Traditional Chinese Medicine Systems Pharmacology (TCMSP).

Molecule Name	OB (%)	DL	Caco-2
Luteolin	36.16	0.25	0.19
(R)-linalool	39.80	0.02	1.33
L-Limonene	38.09	0.02	1.83
Beta-Citronellol	38.89	0.02	1.20

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3-Methyl-3-hepten-2-one	55.53	0.01	1.33
p-coumaric acid	43.29	0.04	0.46
(-)-Citronellal	35.71	0.02	1.34
EEE	45.02	0.00	1.07
OXA	63.21	0.00	0.76
(2S)-2-methylbutan-1-ol	81.23	0.00	1.06
Citral	22.52	0.02	1.33
Geraniol	23.93	0.02	1.19
Neral	19.48	0.02	1.36
Nerylacetate	25.94	0.04	1.28
Mycrene	24.96	0.02	1.84
Heriguard	11.93	0.33	-1.03
Terephthaldehyde	11.93	0.33	-1.35
Caffeic acid	25.76	0.05	0.21
Methose	1.68	0.03	-1.80
Octacosanol	10.70	0.41	1.48
n-Hexacosanol	10.95	0.35	1.42
Citraurin beta	20.53	0.56	1.07
Dotriacontanol	10.25	0.46	1.48
luteolin-7-O-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranside	3.84	0.73	-2.19

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Triacontanol	11.19	0.46	1.42
Isoorientin	23.30	0.76	-1.35
Cymbopogne	29.95	0.77	1.43

OB, Oral bioavailability; DL, Drug-likeness; Caco-2; Caco-2 permeability.

4.2 Target Genes Identification of LUT, GEM and Pancreatic cancer

In LUT, a total of 296 target genes were retrieved from DGIdb, CTD, SwissTargetPrediction and ChemMapper databases. From Drugbank, DgIdb and CTD databases as well, a total of 352 target genes of GEM were retrieved. As for the target genes related to pancreatic cancer, a total of 421 genes were acquired from MalaCards and OMIM databases after removing duplication and combining the results from both databases.

4.3 Screening Potential Pancreatic Cancer-Related Targets of LUT and GEM

A Venn diagram was created using an online tool to represent the potential pancreatic cancer-related target genes of LUT and GEM (Figure 4.1). The diagram revealed 67 LUT-intersection (Figure 4.1(a)), 89 GEM-intersection (Figure 4.1(b)), 77 between LUT- and GEM-intersection (Figure 4.1(c)), and 114 LUT+GEM-intersection target genes against pancreatic cancer and also identified 42 target genes in shared biotargets-intersection of LUT and GEM against pancreatic cancer (Figure 4.1(d)). Among those intersections, four intersections which are LUT-intersection, GEM-intersection, LUT+GEM-intersection, and shared biotargets-intersection of LUT and GEM were further used to conduct the analyses.

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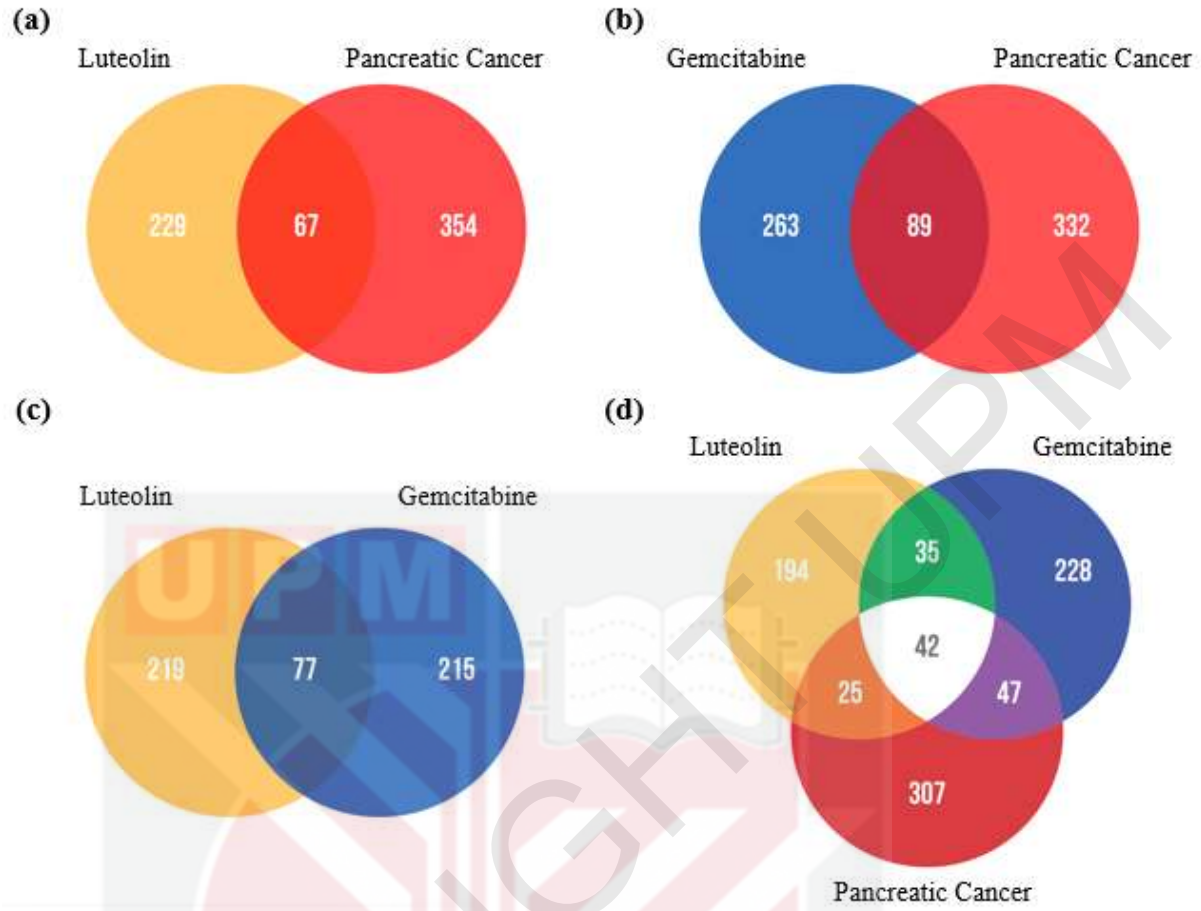

Figure 4.1: The Venn diagram highlighted the intersecting target genes between LUT and GEM against pancreatic cancer. Venn diagram represented **(a)** 67 target genes in LUT-intersection against pancreatic cancer**, (b)** 89 target genes in GEM-intersection against pancreatic cancer, **(c)** 77 target genes between LUT- and GEM-intersection, **(d)** 114 target genes in LUT+GEM-intersection (orange, white, purple) and 42 target genes in shared biotargets-intersection of LUT and GEM (white) against pancreatic cancer.

4.4 Protein-Protein Interaction (PPI) Network

Four intersections which are LUT-intersection, GEM-intersection, LUT+GEM-intersection, and shared biotargets-intersection were investigated in this study. The PPI network of these four intersections were constructed using the STRING

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database (Figure 4.2). From the PPI network presented below, there are 67 nodes and 346 edges of LUT-intersection (Figure 4.2(a)), 88 nodes with one disconnected node (*MIR24-2*) and 425 edges of GEM-intersection (Figure 4.2(b)), 113 nodes with one disconnected node (*MIR24-2*) and 628 edges of LUT+GEM-intersection (Figure 4.2(c)), and 42 nodes and 206 edges of shared biotargets-intersection (Figure 4.2(d)) against pancreatic cancer. The nodes represent the target genes while the edges represent the interaction between them. The networks showed that these target genes have complex interactions between each other.

Several potential pancreatic cancer-related target genes of LUT and GEM have been identified in the network and were considered as the hub genes based on the degree, closeness and betweenness values. Hub genes are highly interconnected with nodes in the network and possess high biological importance in a certain disease. Based on Figure 4.3, *TP53* and *AKT1* were identified as the hub genes that overlapped between the four intersections. *VEGFA* was identified as the only hub gene that overlapped between the three intersections except shared biotargets-intersection, while in shared biotargets-intersection, *BCL2L1* was the only hub gene that is not targeted by the other three intersections. *MYC* was only found in GEM-intersection. *EGFR* together with *VEGFA, TP53* and *AKT1* were identified as hub genes that overlapped between LUT+GEM- and GEM-intersections. *STAT3* and *CCND1* together with *TP53* and *AKT1* were identified as the hub genes that overlapped between GEM- and shared biotargets-intersections. The other three genes which are *CASP3, PTEN, MAPK3* were found to be the hub genes that overlapped between LUT- and shared biotargets-intersections against pancreatic cancer.

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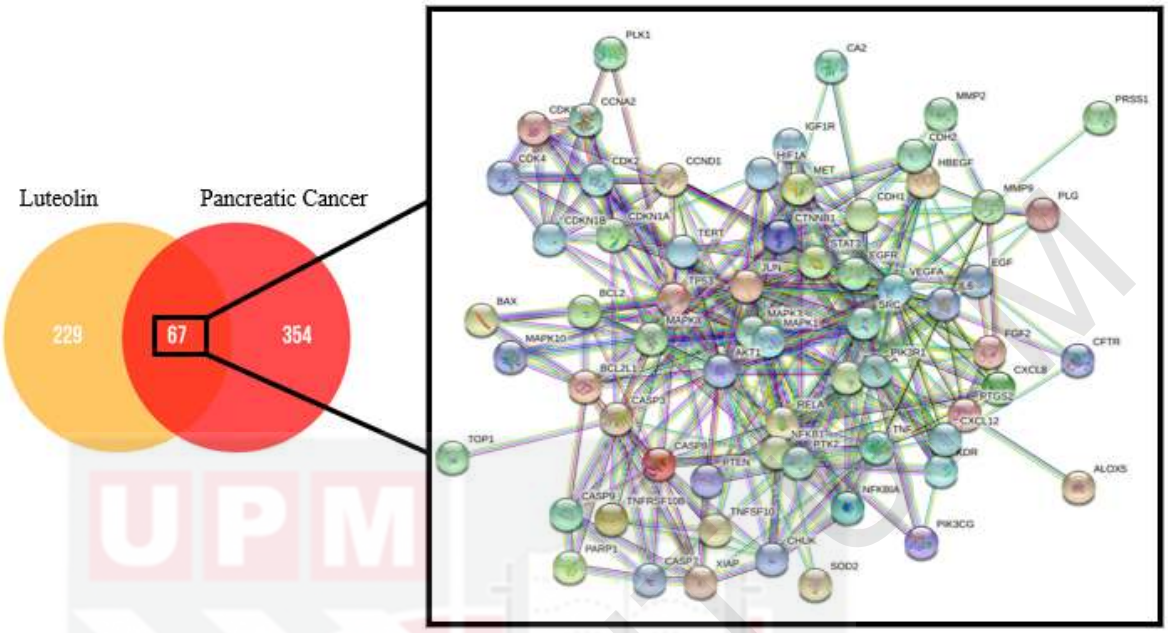
(a)



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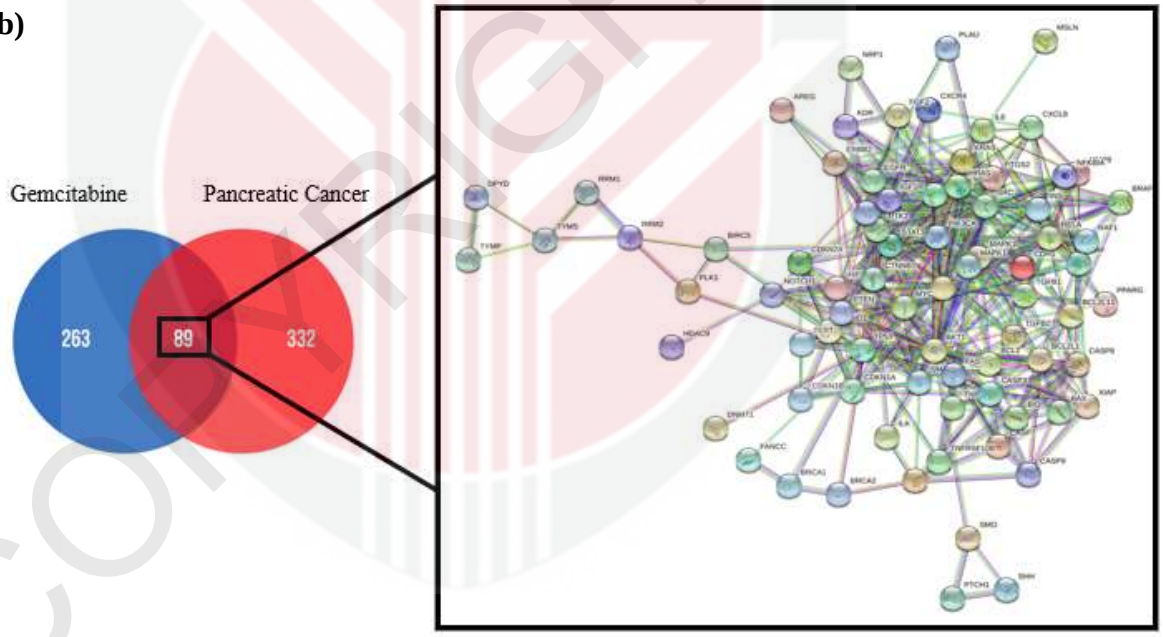

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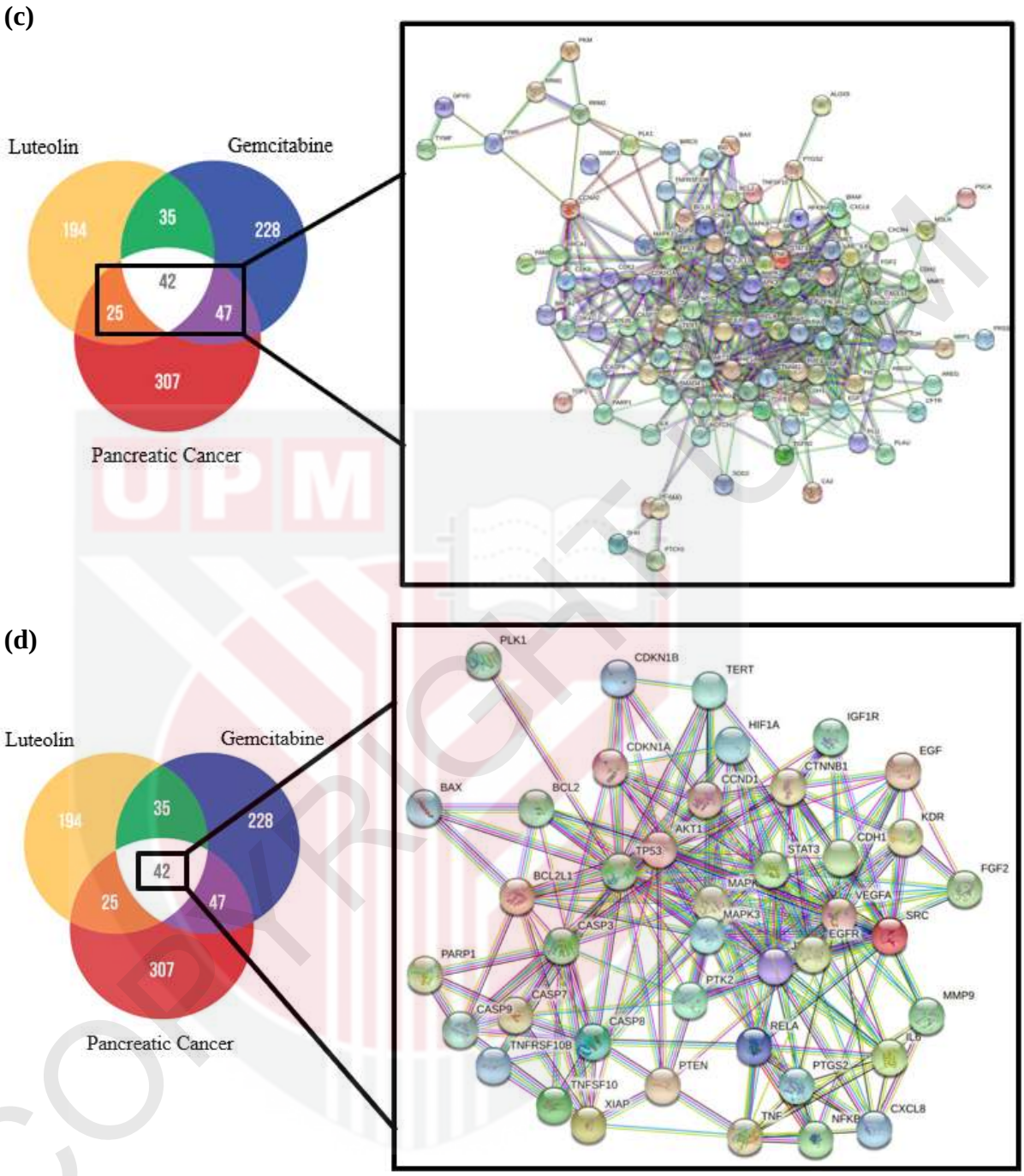

Figure 4.2: Target genes of LUT, GEM and their combination against pancreatic cancer using STRING database. The diagram showed the Venn diagram and PPI network of **(a)** LUT-intersection target genes against pancreatic cancer, **(b)** GEM-intersection target genes against pancreatic cancer, **(c)** LUT+GEM-intersection target genes against pancreatic cancer, and **(d)** shared biotargets-intersection against pancreatic cancer.

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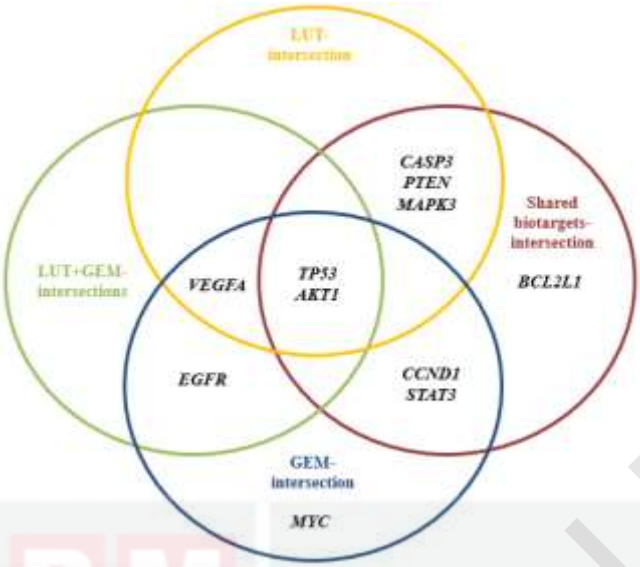

Figure 4.3: Hub genes derived from the PPI network based on degree, closeness and betweenness values. *TP53* and *AKT1* are the hub genes for the combination of LUT and GEM against pancreatic cancer.

4.5 Compound-Target Network and Analysis

The compound-target network (Figure 4.4) of LUT and GEM as well as the shared biotargets-intersection against pancreatic cancer was constructed using Cytoscape software to see the interaction between the compound and the target genes. The combination network contains 116 nodes and 2600 edges including the 2 compounds which are LUT and GEM, and 114 target genes that constitute the network node. These compounds produced a total of 2600 interactions with target genes. Based on the network analysis, 55 target genes with a degree value more than the average degree of node, 44.828, were classified as the high-degree target genes. High-degree target genes can be defined as the genes with a high number of edges linked to a node and it reflects the level of connectivity within the network. The top 10 high-degree target genes which are *TP53, AKT1, VEGFA, EGFR, MYC, CCND1, CASP3, PTEN, STAT3,* and *HRAS* were listed in (Table 4.2).

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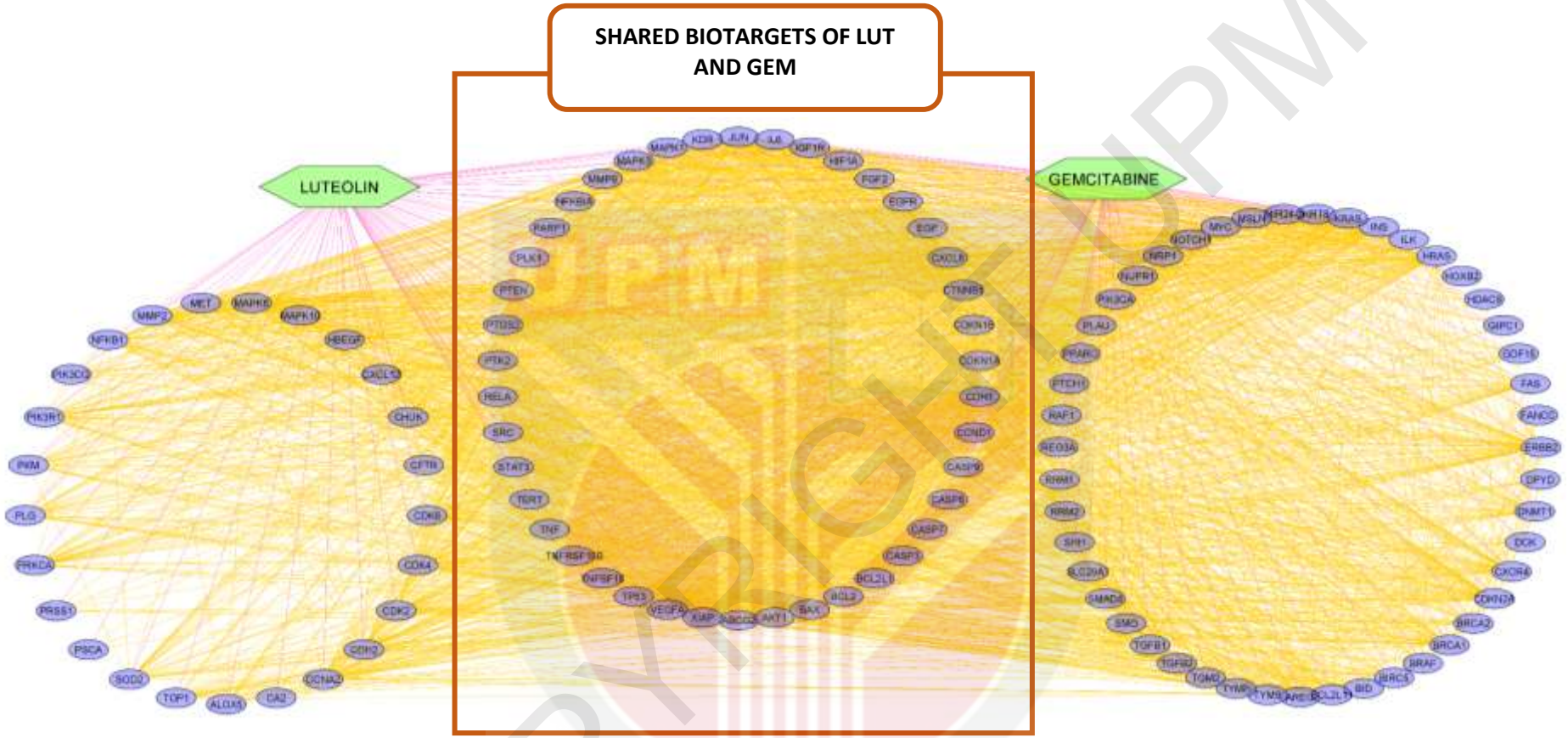

Figure 4.4: The compound-target network of LUT and GEM as well as the shared biotargets-intersection against pancreatic cancer by using Cytoscape. The network contains 116 nodes (2 compounds and 114 potential targets) and 2600 edges. The green and purple nodes represent the compounds and target genes, respectively, while the pink and yellow edges represent the interactions between compound-target and target-target, respectively. The left and right compound-target network are the LUT- and GEM-intersections target genes, respectively, while the middle compound-target network is their combination against pancreatic cancer.

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Table 4.2: Top 10 high-degree target genes of the compound-target network identified from Cytoscape software.

Target genes	Degree
TP53	105
AKT1	93
VEGFA	92
EGFR	91
MYC	89
CCND1	86
CASP3	85
PTEN	85
STAT3	85
HRAS	82

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4.6 Gene Ontology (GO) and Pathway Enrichment Analysis

The possible biological process and signalling pathways in the treatment of pancreatic cancer was further evaluated through enrichment analysis. The DAVID Bioinformatics Resources tool was used to conduct both GO and pathway enrichment analysis. GO enrichment analysis describes the pancreatic cancer-related target genes of LUT and GEM in detail, considering three main aspects, the biological process in which it participates, its molecular function, and the cellular components. The Kyoto Encyclopedia of Genes and Genome (KEGG) pathway enrichment analysis was also performed to identify the underlying pathway of LUT and its combination with GEM on pancreatic cancers. The results of the GO enrichment terms and KEGG pathway analysis are illustrated in (Figure 4.5 and 4.6).

In the GO enrichment analysis of the four intersection networks, three biological processes which are negative regulation of apoptotic process, positive regulation of cell proliferation, and response to drug were highly regulated compared to other processes. This can be seen in Figure 4.5(a), Figure 4.5(b), Figure 4.5(c) and Figure 4.5(d). The top 10 significant enrichment terms represented by molecular function showed that protein binding was highly regulated in LUT-intersection (Figure 4.5(a)), GEM-intersection (Figure 4.5(b)), and LUT+GEM-intersection (Figure 4.5(c)) while identical protein binding was highly regulated in shared biotargets-intersection only (Figure 4.5(d)). In cellular component enrichment analysis, the top 10 significant enrichment terms were mainly involved in the cytosol among the four intersection networks. This can be seen in Figure 4.5(a), Figure 4.5(b), Figure 4.5(c) and Figure 4.5(d).

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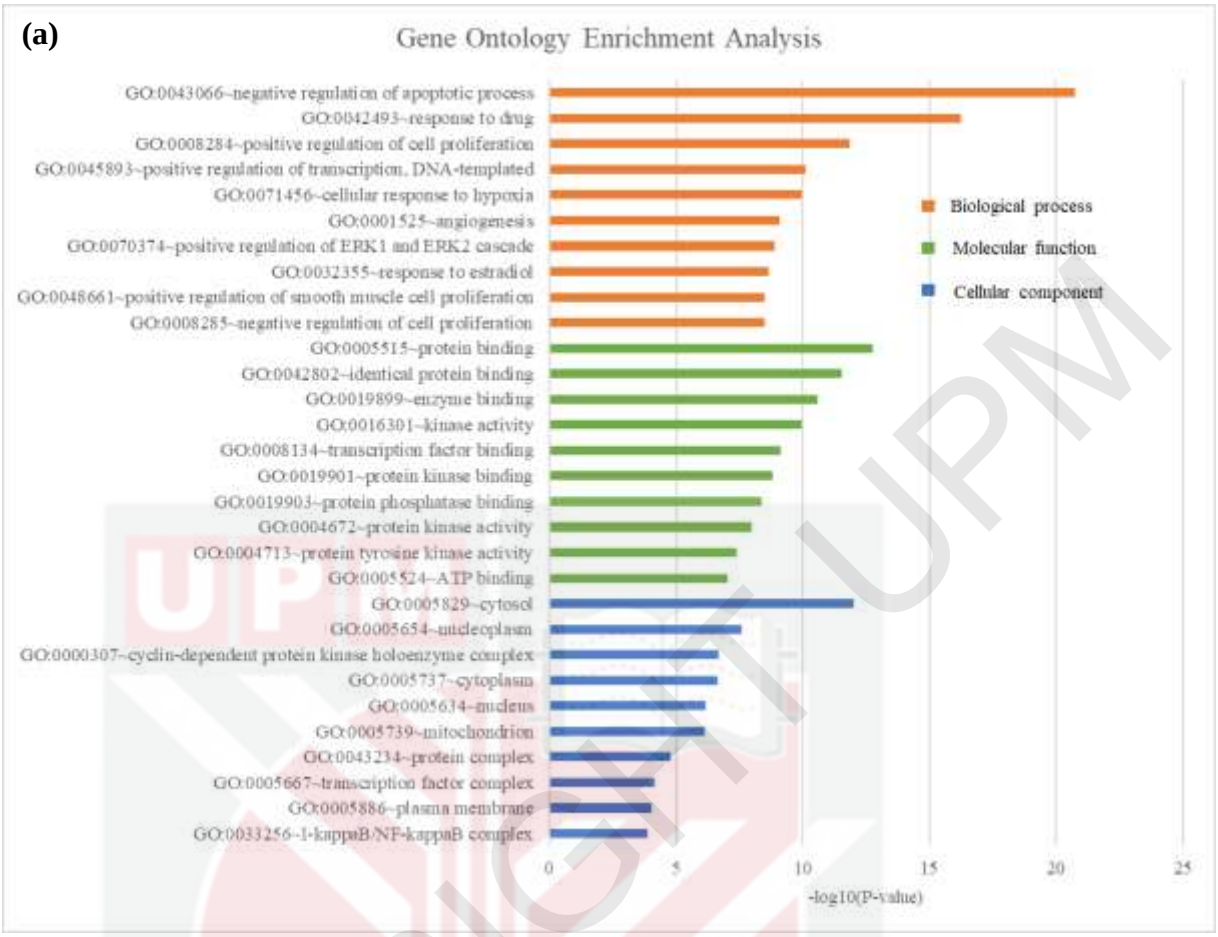

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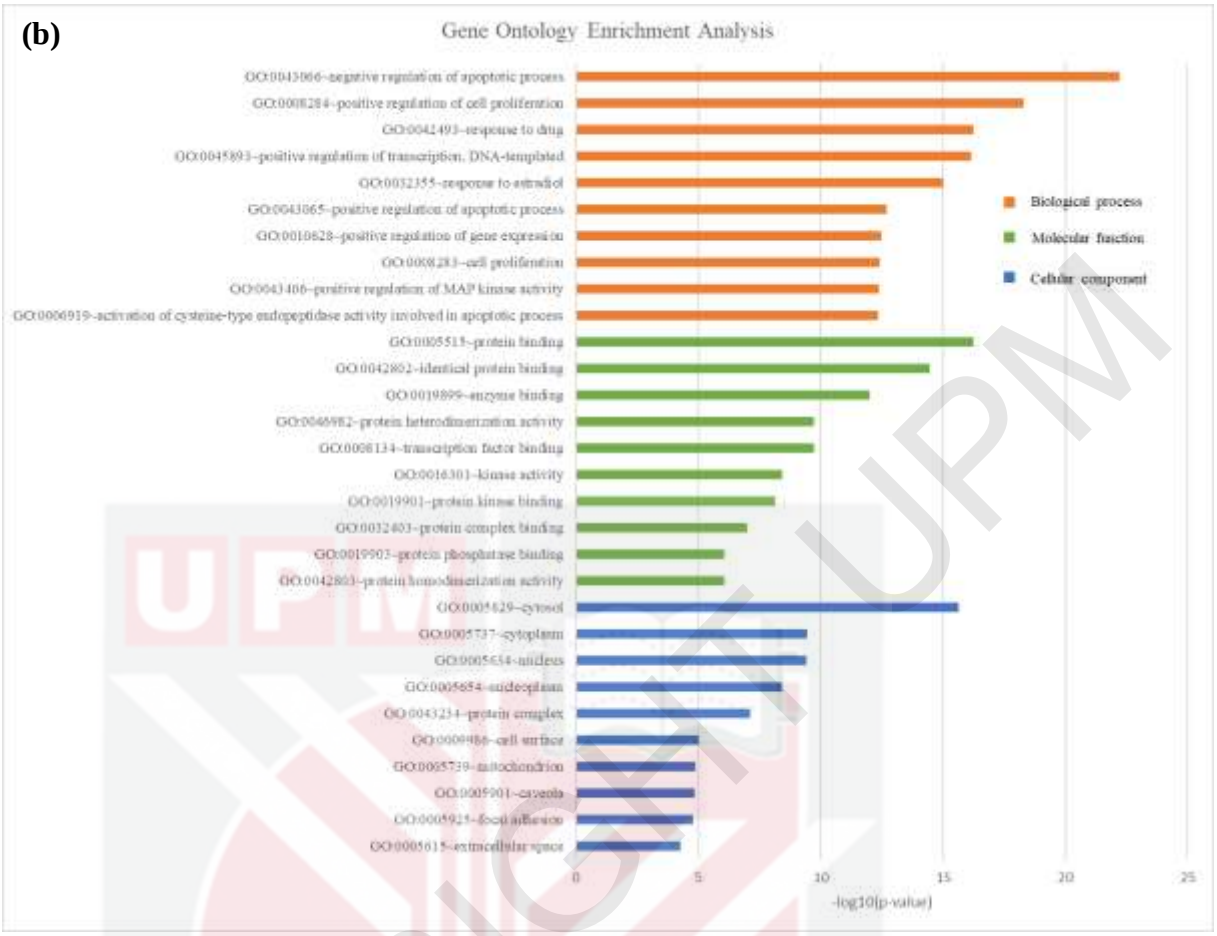

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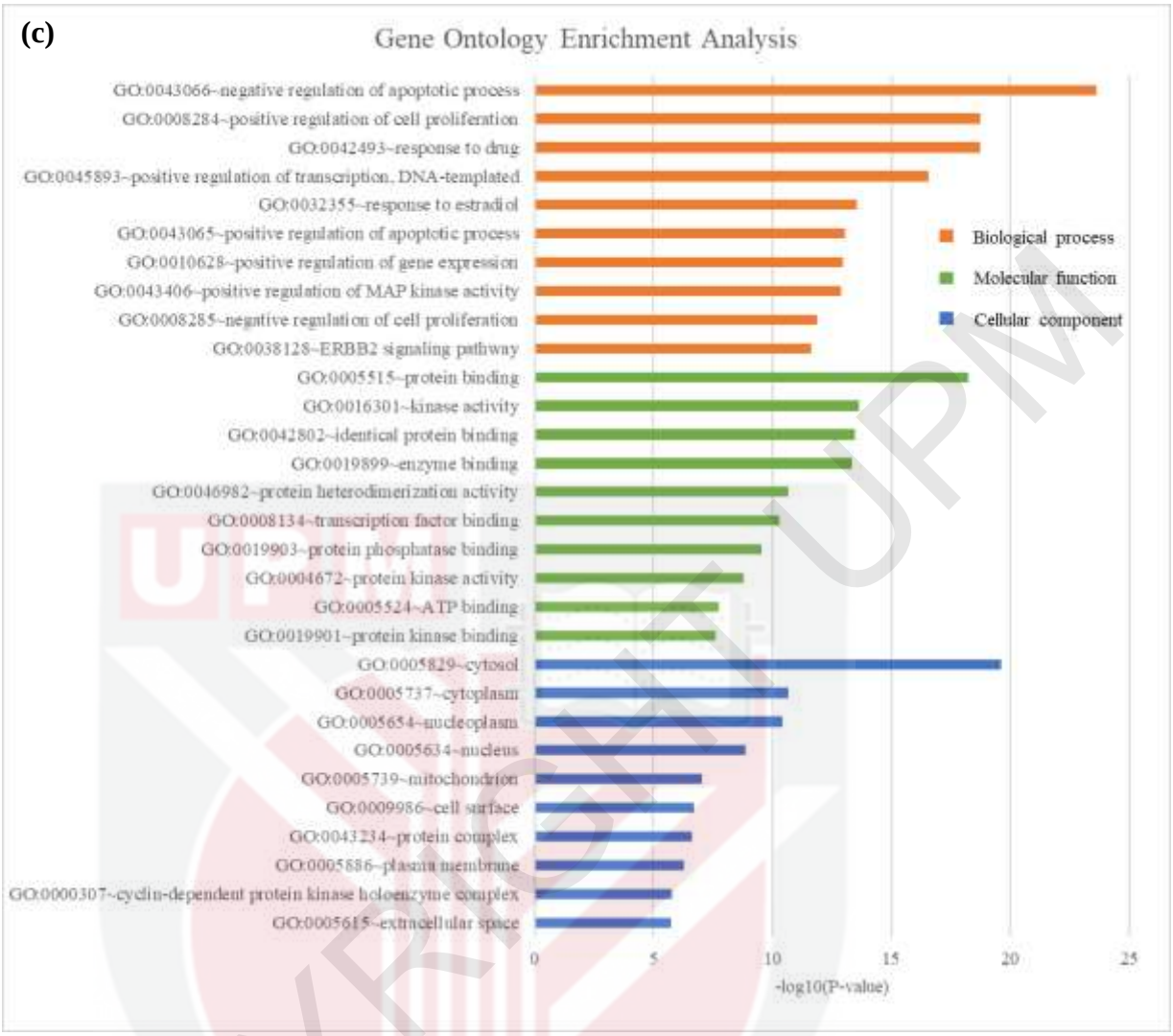

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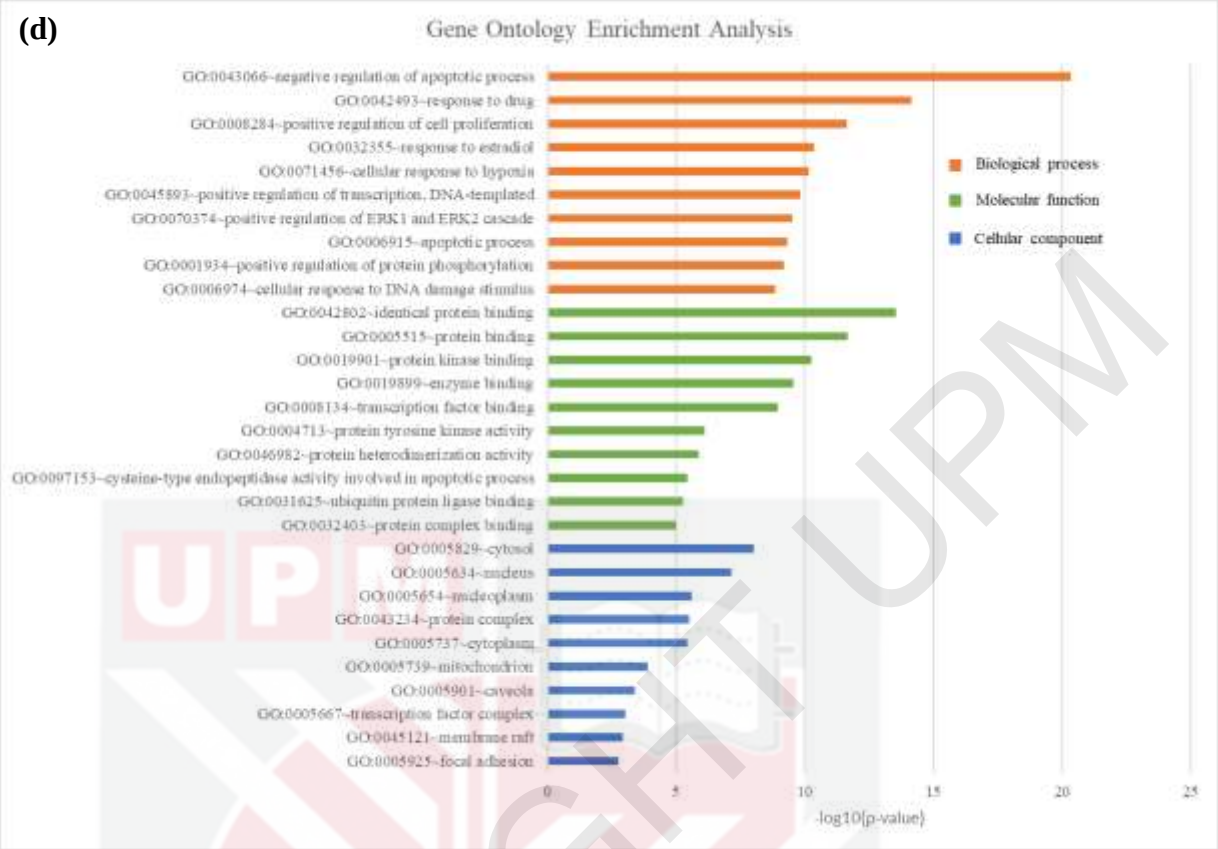

Figure 4.5: GO enrichment terms analysis of LUT, GEM and their combination against pancreatic cancer. The bar chart represents the top 10 significant enrichment terms represented by the biological process, cellular component and molecular function of **(a)** LUT-intersection target genes against pancreatic cancer, **(b)** GEM-intersection target genes against pancreatic cancer, **(c)** LUT+GEM-intersection target genes against pancreatic cancer, and **(d)** shared biotargets-intersection against pancreatic cancer.

In the KEGG pathway analysis of the four intersection networks, pathways in cancer and hepatitis B were highly regulated compared to other pathways. There is also involvement of many cancer-related pathways, including pancreatic cancer. Among the four networks, pancreatic cancer ranked as third in the LUT-intersection (Figure 4.6(a)) and LUT+GEM intersection (Figure 4.6(c)), fifth in the GEM-intersection (Figure 4.6(b)) while as the last rank (tenth) in the shared biotargets-

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intersection (Figure 4.6(d)). A collection of molecular interaction network diagrams for metabolic pathways, signalling pathways, pathways involved in various cellular processes and organism systems, and also pathways associated with pancreatic cancer was shown in (Figure 4.7). The activating point mutations in the *KRAS* and overexpression of *HER-2/neu* occur early, inactivation of the *p16* at an intermediate stage, and the inactivation of *p53, SMAD4,* and *BRCA2* occur relatively late. Mutations in *KRAS* oncogenes activate multiple effector pathways such as PI3K-Akt signalling pathway and MAPK signalling pathway that later promotes proliferation and cell survival and also suppresses the apoptosis process. *HER-2/neu* oncogene is involved in multiple effector pathways as well which are JAK-STAT signaling pathway and VEGF signalling pathway that later promote the formation of new blood vessels which are termed as angiogenesis. *p16* gene plays an important role in regulating the cell cycle where its inactivation will lead to uncontrolled G1/S phase progression and causing the cell to divide rapidly and loss the ability to control replication. The inactivation of *p53* gene will also lead to uncontrolled cell proliferation, and also increased cell survival and causing genomic instability. As for *SMAD4* genes, its inactivation will lead to loss of the growth inhibitory effect of *TGFB,* thus increasing cell survival. *BRCA2* mutations on the other hand 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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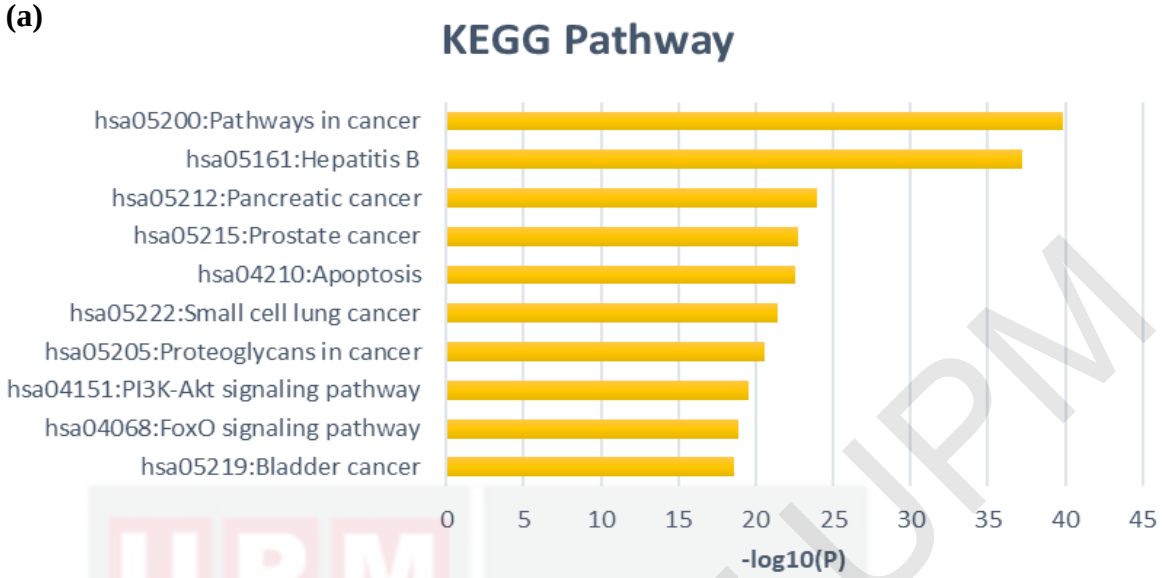

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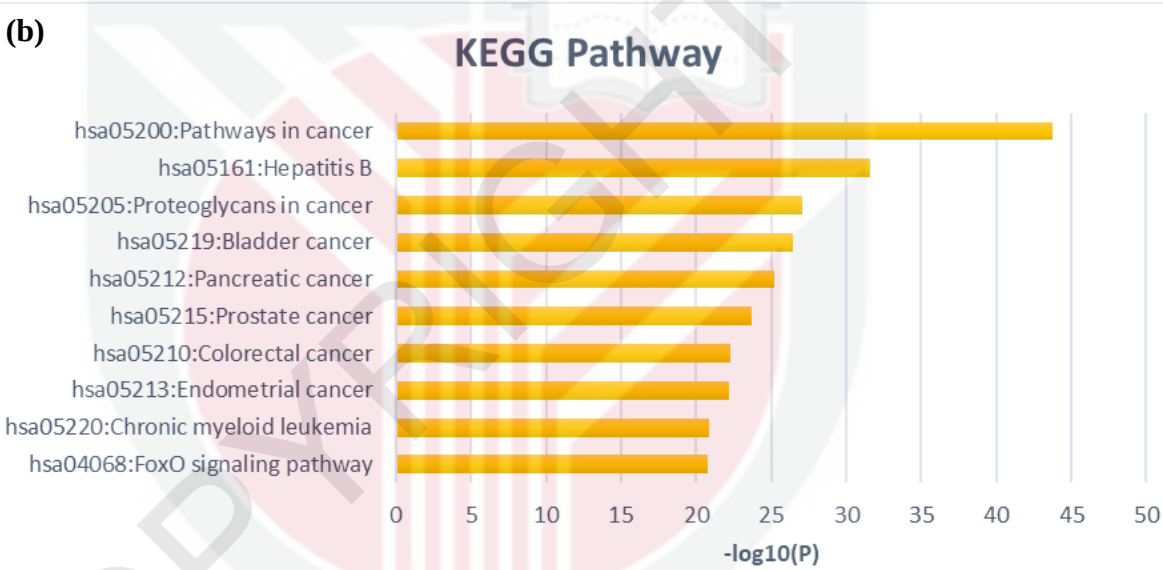

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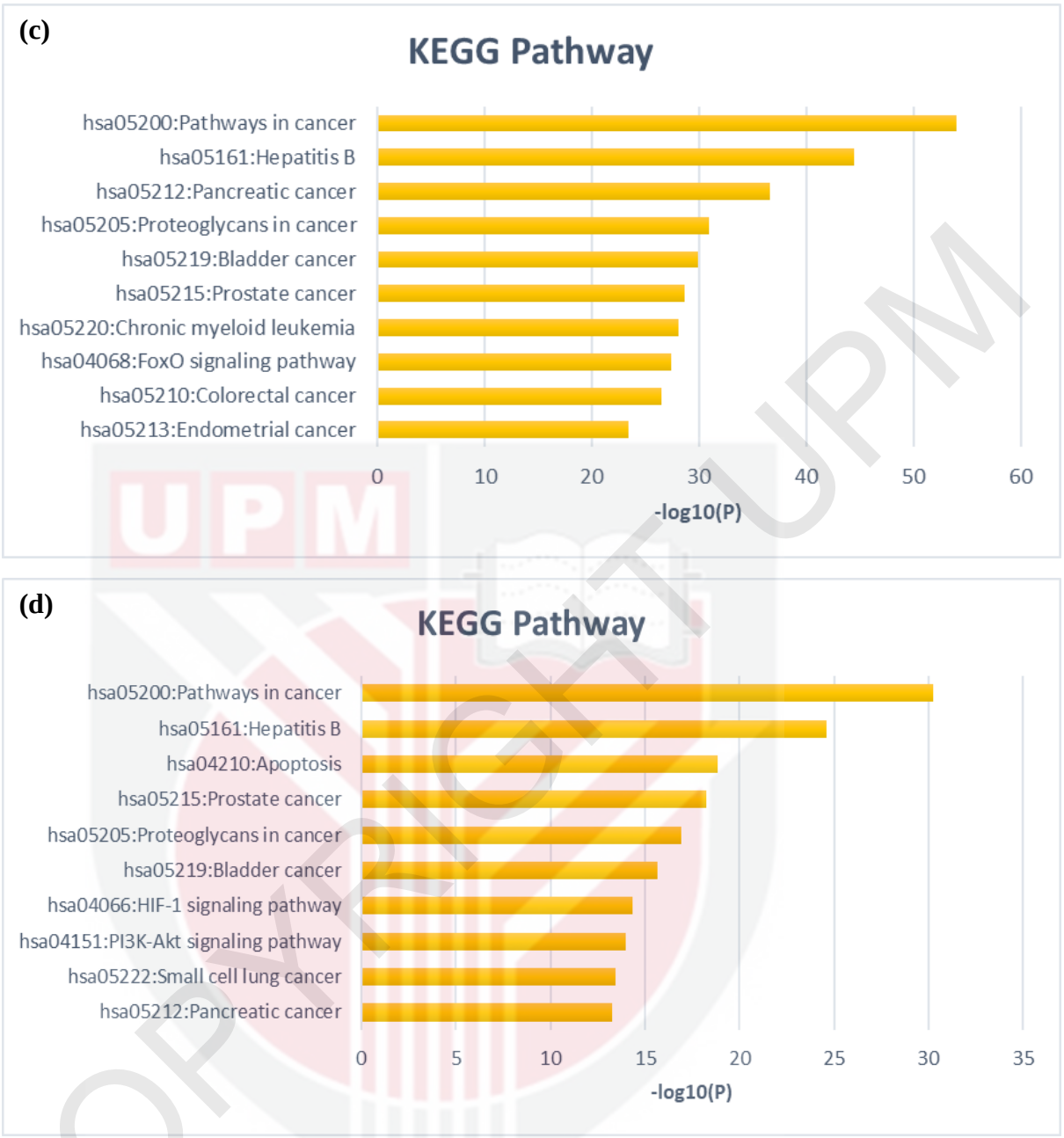

Figure 4.6: The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of LUT, GEM and their combination against pancreatic cancer. The bar chart represents the top 10 KEGG pathway analysis of **(a)** LUT-intersection target genes against pancreatic cancer, **(b)** GEM-intersection target genes against pancreatic cancer, **(c)** LUT+GEM-intersection target genes against pancreatic cancer, and **(d)** shared biotargets-intersection against pancreatic cancer.

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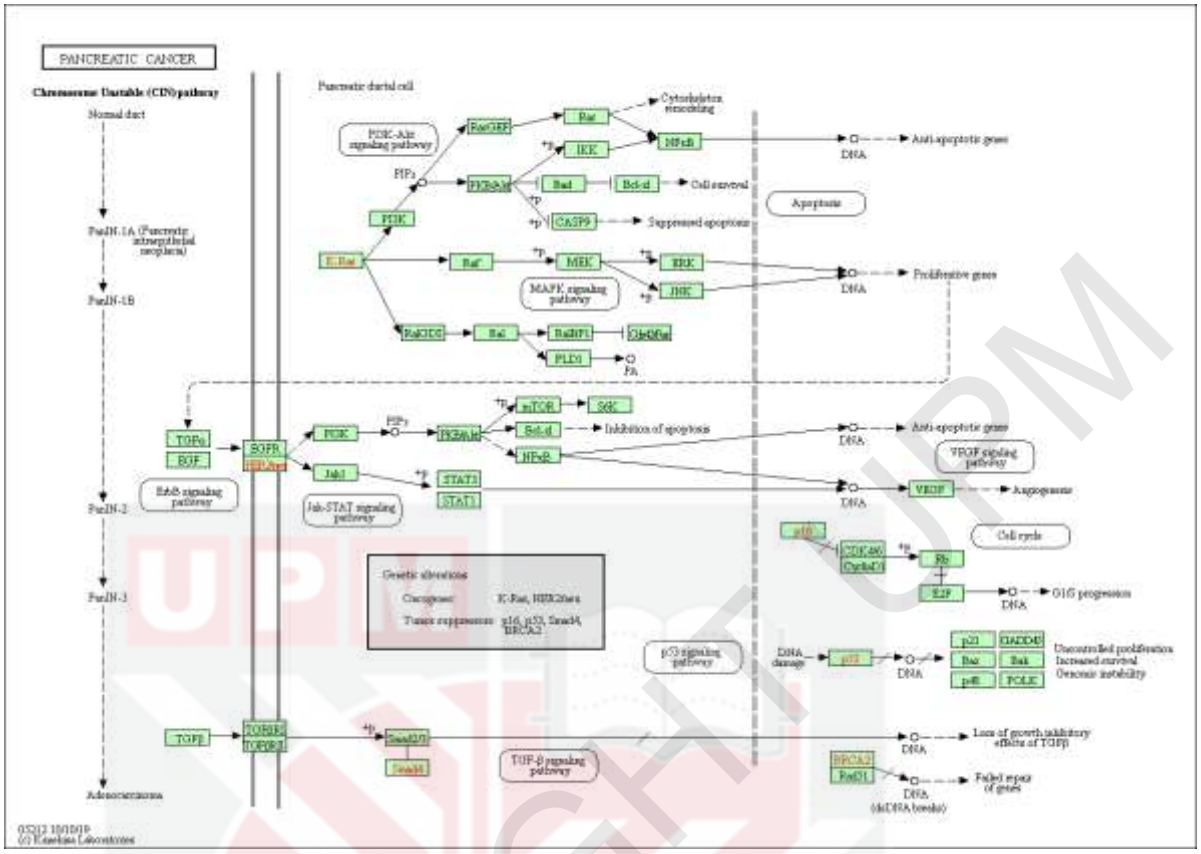

Figure 4.7: Diagram of the pancreatic cancer pathway targeted by the combination of LUT and GEM from the KEGG pathway map. The genes in red text are the target genes of LUT and GEM in pancreatic cancer including the *KRAS* and *HER2/neu* as the oncogenes and *p16, p53, SMAD4* and *BRCA2* as the tumor suppressors genes. The activation of *KRAS* and *HER2/neu* involves 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 to contribute to extensive genomic instability and aneuploidy.

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4.7 Target-Pathway Network and Analysis

The target-pathway network (Figure 4.8) of LUT and GEM as well as the shared biotargets-intersection against pancreatic cancer was constructed using Cytoscape based on the significantly enriched pathways to further find the relations between the target genes and the important pathway involved. The network demonstrated that LUT and GEM as well as the combination of the two exerted its therapeutic effects against pancreatic cancer primarily by regulating the 14 pathways. This network contains 94 nodes and 399 edges including the 80 target genes and 14 pathways that constitute the network node. The target gene produced a total of 399 interactions with the involved pathway. Based on the network analysis, 20 target genes with a degree value more than the average degree of node, 8.489, were classified as the high-degree target genes. The top 10 high-degree target genes are *AKT1*, *TP53*, *MAPK3*, *MAPK1*, *CCND1*, *KRAS*, *RAF1*, *BRAF*, *PIK3CA*, and *RELA* (Table 4.3).

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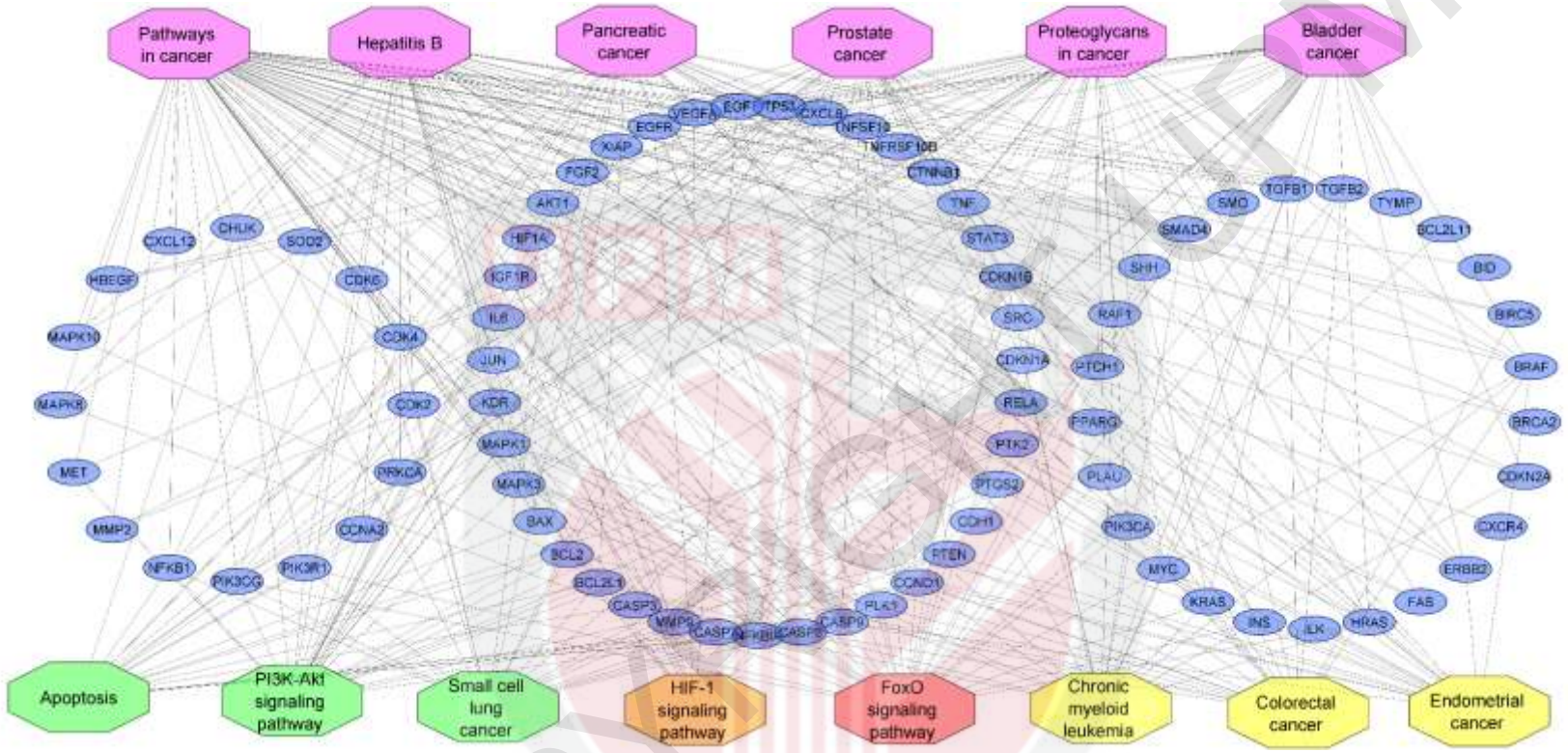

Figure 4.8: The target-pathway network of LUT and GEM as well as the shared biotargets-intersection against pancreatic cancer by using Cytoscape. The network contained 94 nodes (80 target genes and 14 pathways) and 399 edges. The oval and hexagon nodes represent the target genes and pathways, respectively, while the edges represent the interactions between them. The left and right network are the LUT- and GEM-intersections target genes, respectively while the middle network is their combination against pancreatic cancer. The pink hexagons represent the pathways involved in three intersection networks, the green hexagons represent the pathways involved in LUT- and shared biotargets-intersections, the orange hexagons represent the pathways involved in shared biotargets-intersection, the red hexagons represent the pathways involved in LUT- and GEM- intersections and the yellow hexagons represent the pathways involved in GEM- intersection.

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Table 4.3: Top 10 high-degree target genes of the target-pathway network identified from Cytoscape software.

Target genes	Degree
AKT1	13
TP53	12
MAPK3	12
MAPK1	12
CCND1	12
KRAS	10
RAF1	10
BRAF	9
PIK3CA	9
RELA	9

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CHAPTER 5

DISCUSSION

At present, Pancreatic Cancer Action Network (PanCAN) has revealed the increment of the five-year survival rate for pancreatic cancer patients from 6% to 10% from 2014 to 2021. Although pancreatic cancer survival rates have been proven to have improvement, this cancer remained to be considered as the largely incurable type of disease and often associated with poor prognosis in cancer diagnosis. This is because pancreatic cancer is usually detected in a patient at a very late stage. Furthermore, an effective drug treatment of pancreatic cancer is still not extensively studied. Traditional Chinese Medicine (TCM) knowledge has been widely used as an important source of effective therapeutic drugs. Therefore, in this study, a network pharmacology approach has been applied to study ancient TCM and also modern drug discovery to find a cure for the disease.

Gemcitabine (GEM), a standard choice of treatment for locally advanced and metastatic pancreatic cancer has been used for more than 15 years as a chemotherapeutic agent. According to a previous study, GEM alone was found to exhibit its anti-cancer activity against pancreatic cancer by inducing apoptotic process. In order to respond to the cellular stress in cancer cells, GEM triggers apoptosis by activating p38 mitogen-activated protein kinase (MAPK) (De Sousa Cavalcante & Monteiro, 2014). Another study also found that GEM induced programmed cell death in PK1 and PK8 human pancreatic cancer cells by inhibiting the PI3K-PKB/Akt signalling pathway (Ng et al., 2000). GEM also induces apoptosis by down-regulating the antiapoptotic PAP gene, and up-regulating the pro-apoptotic TP53INP1 gene and GSK-3β^{ser9} protein. In other words, GEM induced programmed cell death in pancreatic cancer cells by inhibiting the PAP gene, and activating TP53INP1 and

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GSK-3β^{ser9} protein (Jiang et al., 2006). However, in recent years, GEM resistance development has been commonly discovered by most researchers. This could alter the efficacy of the drug against pancreatic cancer. Hence, this current study combined a modern drug with herbal medicine in order to help future researchers developing an effective drug specific for pancreatic cancer.

Through Traditional Chinese Medicine Systems Pharmacology (TCMSP) and Traditional Chinese Integrated Medicine Database (TCIMD), 27 candidate bioactive compounds of lemongrass were collected. Among those, luteolin (LUT) was found to be the only bioactive compound of lemongrass after undergo absorption, distribution, metabolism, and excretion (ADME) screening with the value of 36.16%, 0.25 and 0.19 for oral bioavailability, drug-likeness and Caco-2 permeability respectively. LUT, 3′,4′,5,7-tetrahydroxyflavone, is a member of the flavone subclass of flavonoid and was previously known to exhibit anti-cancer effects in various cancer cells including breast cancer, lung cancer and leukaemia (Johnson et al., 2015). Recent study also revealed that LUT is involved in the pancreatic cancer management by inducing programmed cell death and cell cycle arrest, and suppressing the protein phosphorylation and signalling. (Imran et al., 2019).

From the protein-protein interaction (PPI) network, there are several genes recognized as the hub genes. Hub genes are highly interconnected with nodes in the network and possess high biological importance in a certain disease (Wu et al., 2020). In this study, *TP53, AKT1, VEGFA* and *EGFR* were identified as the hub genes in the network of LUT+GEM-intersection target genes against pancreatic cancer. *TP53* is a tumour suppressor gene that regulates the G1-S cell cycle checkpoint, is involved in maintenance of G2-M arrest, and induces apoptosis. When this gene is mutated, the cells will continue to survive and divide abnormally, thus leading to the malignant

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progression of the cancer (Hidalgo, 2011). *AKT1* is an oncogene that has the ability to control cell cycle arrest, inhibit apoptosis and promote the formation of new blood vessels (Zavoral et al., 2011). Vascular endothelial growth factor A (VEGFA) is a protein that is encoded by the *VEGFA* gene. This gene plays an important role in inducing invasion and migration of pancreatic cancer cells since it promotes angiogenesis and significantly enhances the motility of cancer cells (Costache et al., 2015). *EGFR* is a receptor tyrosine kinase that is abnormally activated and over-expressed in pancreatic cancer. Over-expression is often associated with more advanced disease, presence of metastases and poor survival (Oliveira-Cunha et al., 2011). Considering *AKT1, VEGFA* and *EGFR* are the genes that are involved in the development of pancreatic cancer, inhibiting these 3 genes could be a promising therapeutic target of the disease.

As for the network of LUT-intersection target genes against pancreatic cancer, this study also identified *TP53, AKT1* and *VEGFA* as the hub genes with 3 additional genes which are *PTEN, CASP3* and *MAPK3*. *PTEN* plays an important role in blocking tumour angiogenesis. Hence, it is regarded as a tumour suppressor gene. Mutation of this gene would enhance the stimulation of angiogenesis, leading to a high level of VEGF secretion (Ma et al., 2009). *CASP3* is a pro-apoptotic protein which plays a role in the apoptotic process. Along with its function in promoting apoptosis, *CASP3* is also involved in cell cycle regulation, cleaving the cyclin-dependent kinase inhibitors p21 and p27, which eventually inhibit the cell cycle (Radziszewska et al., 2009). Mitogen-activated protein kinase 3 (*MAPK3*) is an enzyme encoded by the *MAPK3* gene. It is involved in a signalling cascade that mediates various cellular processes such as proliferation, differentiation and cell cycle progression (Wang et al., 2019). Therefore, up-regulating *MAPK3* gene and down-regulating *PTEN* and *CASP3* genes

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could interrupt signalling cascades, suppress angiogenesis and increase apoptosis, thus reducing the cancer cells' survival. According to other study previously, LUT was found to significantly reduced the expression of nuclear GSK-3β and NF-κB p65 in BxPC-3 human pancreatic cancer cells and in other cancer, such as breast cancer, LUT was shown to significantly inhibit the proliferation and suppress the expression of *STAT3, EGFR, AKT,* and *ERK1/2* in MCF-7 breast cancer cells (Imran et al., 2019).

In the GEM-intersection target genes against pancreatic cancer, this study also identified *TP53, AKT1, VEGFA,* and *EGFR* as the hub genes with 3 additional genes which are *CCND1, STAT3* and *MYC. CCND1* is a well-known oncogene that is highly expressed in pancreatic cancer and significantly associated with poor prognosis. Previous study mentioned that interrupting the *CCND1* gene could influence pancreatic cancer development by affecting cell growth and metastasis *in vitro* and *in vivo* (Chen et al., 2019). *MYC* is also an oncogene that induces continual tumour growth by increasing DNA replication and transcription, cell proliferation and growth, protein synthesis and metabolism (Hessmann et al., 2016). *STAT3* is a transcription factor encoded by the *STAT3* gene. It regulates VEGF expression and pancreatic cancer angiogenesis, growth, and metastasis (Wei et al., 2003). When this gene was mutated, the pancreatic cancer cells growth was significantly halted. Since *CCND1, MYC* and *STAT3* are the genes that promote pancreatic cancer formation and development, targeting these 3 genes could be a potential therapeutic target in controlling the growth of the disease.

On the other hand, when LUT and GEM were combined to treat pancreatic cancer, *TP53, CASP3, AKT1, CCND1, PTEN, MAPK3* and *STAT3* were also identified as the hub genes with 1 additional gene which is *BCL2L1*. In contrast to *CASP3, BCL2L1* is an important anti-apoptotic protein that aids in the survival of

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differentiating pancreatic cancer cells (Loo et al., 2020). Therefore, considering the *BCL2L1* gene function, down-regulating this gene could increase apoptosis, thus limiting cancer cell survival. In addition, since *TP53* and *AKT1* were targeted in the four networks, these genes might have major biological importance in treating pancreatic cancer.

Moreover, this study also suggests that the combination treatment of LUT and GEM might have a better effect in treating pancreatic cancer compared to the treatment of LUT alone and GEM alone. This can be seen where the combination of LUT and GEM can target other important functional genes that are not present in LUT alone and GEM alone. Therefore, the treatment might be an effective treatment since both LUT and GEM can work together to target more genes in pancreatic cancer.

Furthermore, the possible mechanism of action and signalling pathways in the treatment of pancreatic cancer was further evaluated through enrichment analysis. In the gene ontology (GO) enrichment analysis, the most enriched biological processes of the target genes of LUT as well as its combination with GEM are involved in the negative regulation of apoptotic process and positive regulation of cell proliferation. It has been reported that failure in activating the apoptotic process and abnormal proliferation of cells could lead to the development of cancer. Therefore, by targeting the genes involved in the process that prevent apoptotic process and increase cell proliferation, the cancer cells would probably reduce in number, thus changing a pancreatic cancer to a curable disease. In the KEGG pathway analysis, the most enriched pathways of the target genes of LUT as well as its combination with GEM are cancer-related pathways, which include pancreatic cancer, bladder cancer and prostate cancer where many targets were involved in either multiple cancer pathways. There are also other pathways which are proteoglycans in cancer and hepatitis B where

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many targets are also involved. These results revealed the “multi-target” and “multi-pathway” pharmacological theories of TCM. In this study, pancreatic cancer is the main focus. The KEGG pathway analysis result showed that pancreatic cancer was highly regulated and ranked as the top 10 pathways when LUT is administered together with GEM. This indicates that the combination treatment of LUT and GEM could be an effective approach in treating pancreatic cancer. Nevertheless, further *in vivo* and *in vitro* experimental validation needs to be conducted to verify these computational findings.

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CHAPTER 6

CONCLUSION, LIMITATION AND RECOMMENDATION

In conclusion, this study was the first to predict the targets of the bioactive compound of lemongrass and combined with gemcitabine against pancreatic cancer and theoretically evaluate the underlying mechanism of their potential anti-pancreatic cancer effects. There are several targets of luteolin, gemcitabine and their combinations that are closely associated with the development of pancreatic cancer including *TP53*, *AKT1*, *VEGFA*, *EGFR*, *MYC*, *CCND1*, *STAT3*, *PTEN*, *CASP3*, *MAPK3*, and *BCL2L1*. These genes were functionally enriched in multiple pathways. Therefore, it is believed that the potential mechanism of its anti-cancer effect on pancreatic cancer is a synergy of multi-target and multi-pathway efforts. Previously, it has been reported that gemcitabine treatment alone was commonly associated with the chemoresistance and combination therapy with other drugs could result in undesirable side effects. Hence, this current study was conducted and has revealed that the effect of gemcitabine will be enhanced when in combination with luteolin as they can target genes that are not targeted by luteolin alone and gemcitabine alone in pancreatic cancer. It is suggested that in the future, this combination can be applied to develop an effective pancreatic cancer drug treatment. However, this research was just a presumptive approach, representing a limitation of the present study that requires further investigation involving experimental validation in the future.

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APPENDICES

APPENDIX I

Identification of the Potential Bioactive Compounds in Lemongrass

- Go to TCMSP database https://www.tcmspw.com/tcmsp.php, choose herb name, insert the Chinese Pinyin name of lemongrass: *Xiang mao*, and click search

- The molecule name and value for each parameter will be shown

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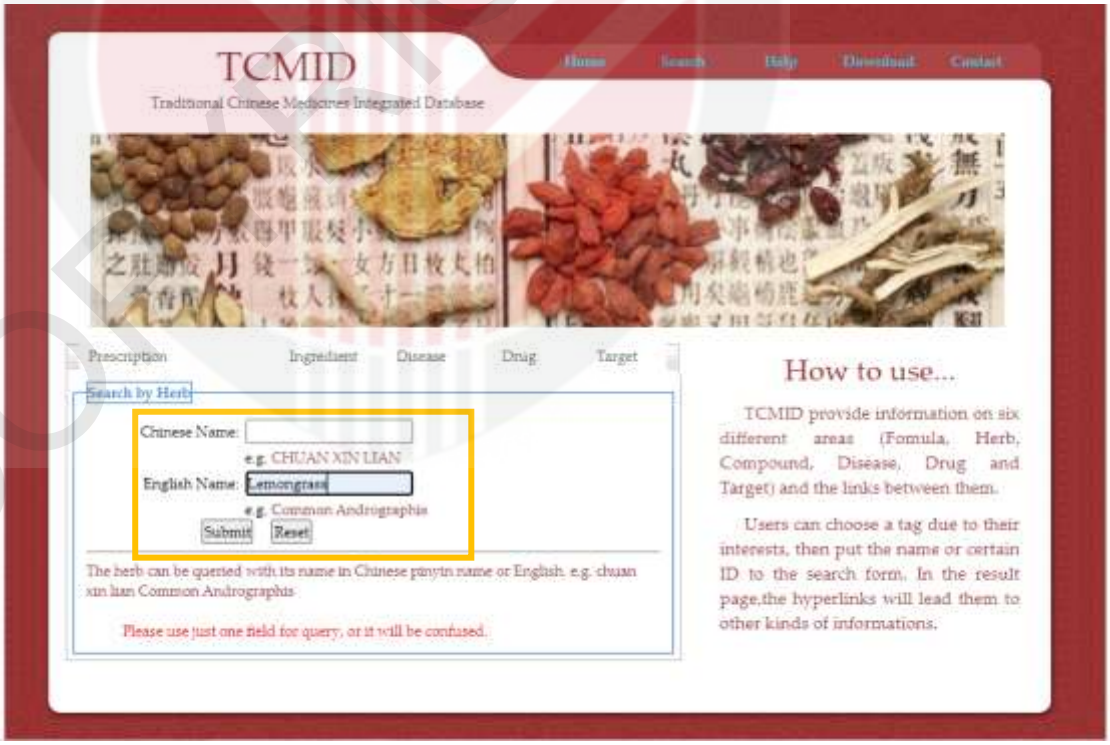
- Go to TCMID website http://119.3.41.228:8000/tcmid/ and click search

- Choose herb, insert lemongrass in the English Name column and click submit



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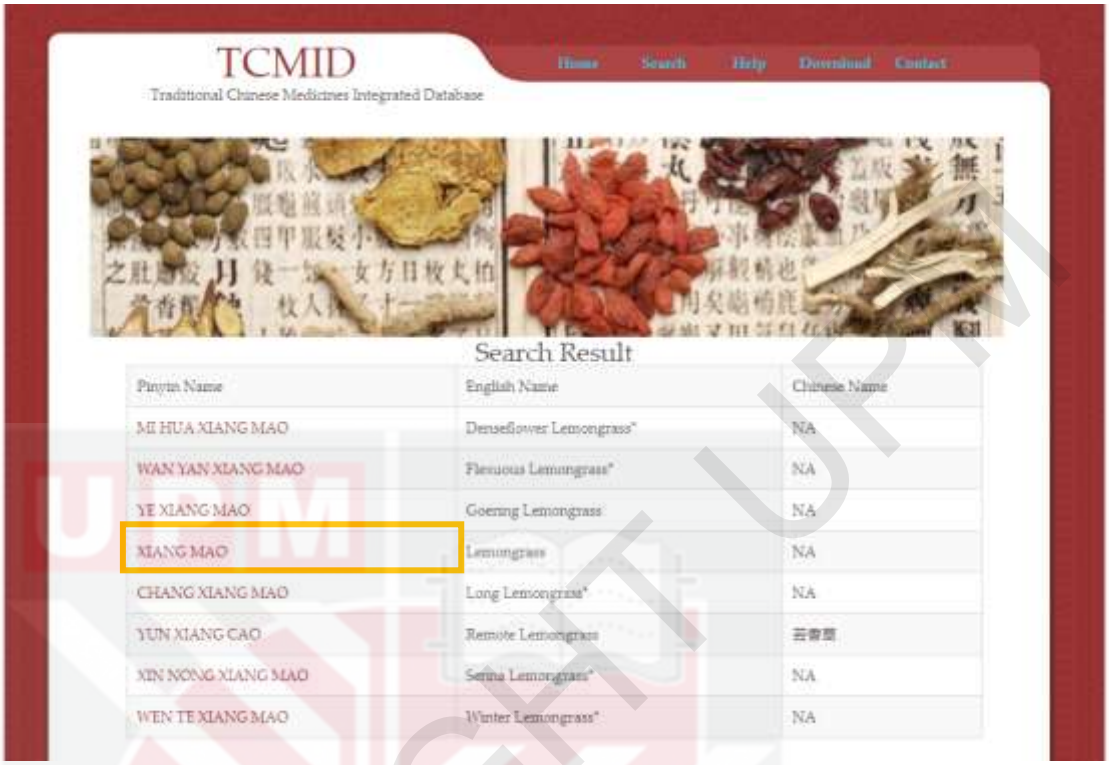
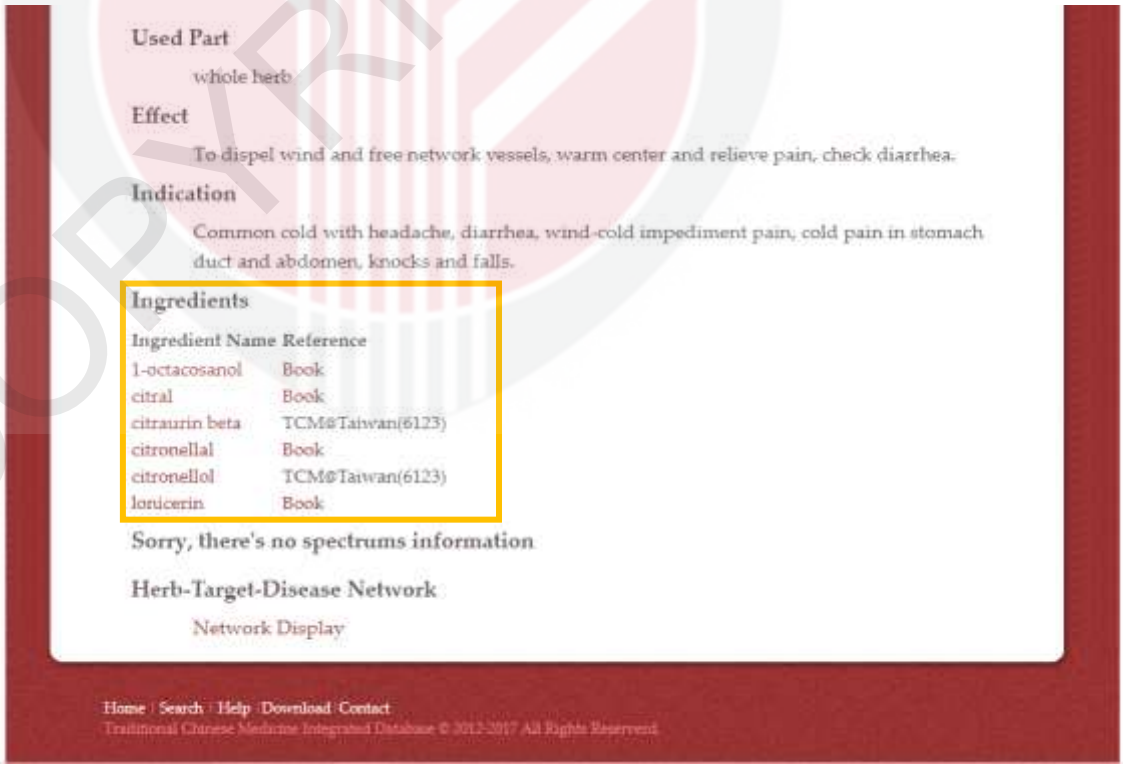
- Select the Pinyin Name as Xiang Mao



- Scroll down to see the list of ingredient name of lemongrass



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- To get the value of oral bioavailability (OB), drug-likeness (DL) and Caco-2 permeability for compound from TCMID, go to https://www.tcmspw.com/tcmsp.php, select Chemical name, insert the compound name, and click search

- Select the molecule name as Citral

- The information related to the compound will be shown including the value of oral bioavailability (OB), drug- likeness (DL) and Caco-2 permeability

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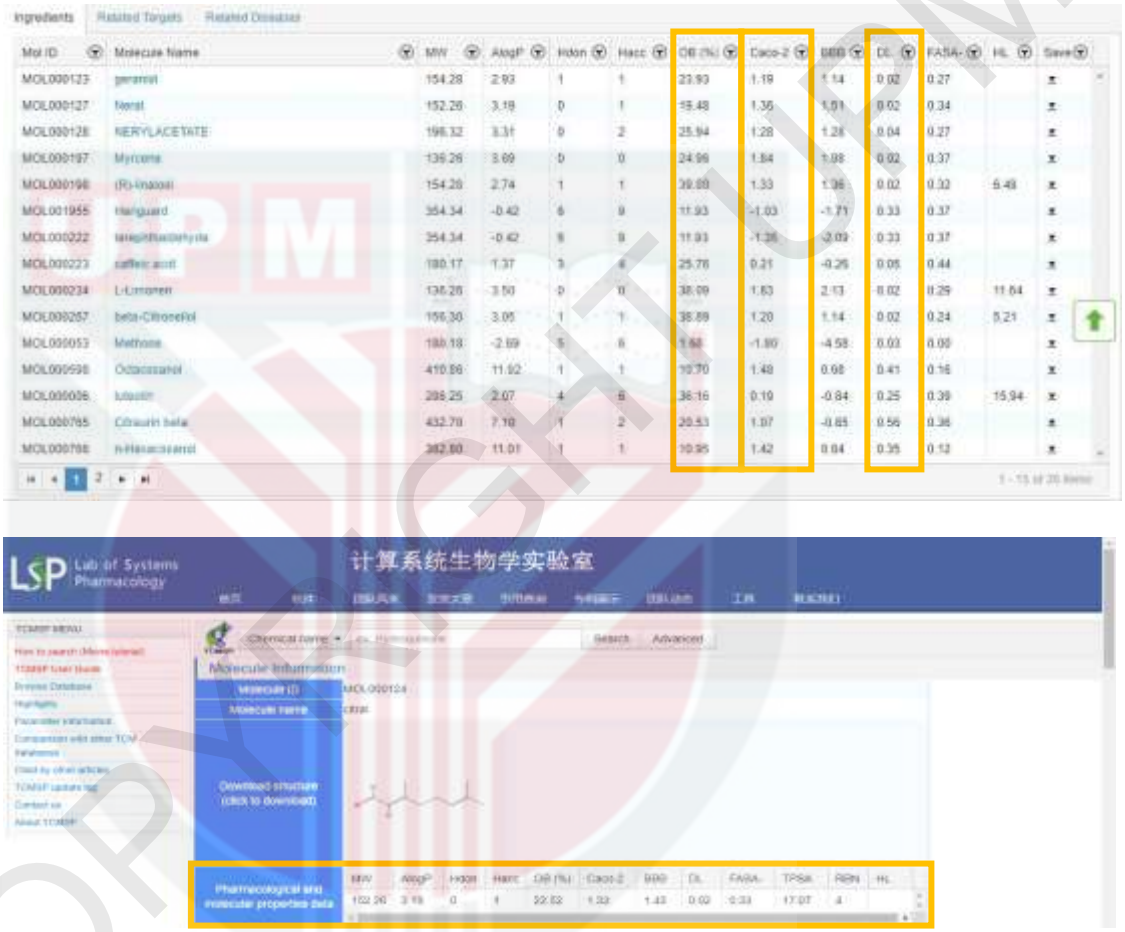
APPENDIX II

In silico Screening of Bioactive Compound of Lemongrass

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



- Compound that met the criteria of OB ≥ 30%, Caco-2 permeability ≥ −0.4 and DL ≥ 0.18 were considered as the active compound of lemongrass and those that does not met the criteria were excluded from the screening process

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APPENDIX III

Target Identification

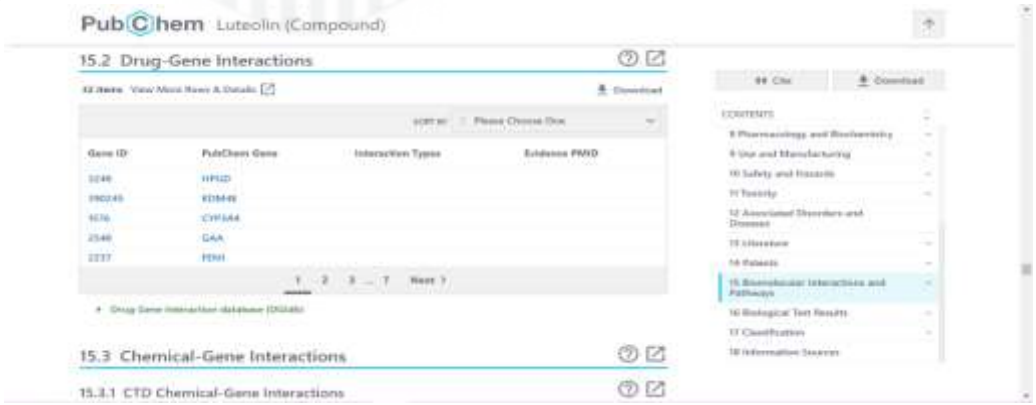
1. Target Genes of Lemongrass Bioactive Compound

- **PubChem: https://pubchem.ncbi.nlm.nih.gov/**

  - Insert the compound name

  - Choose PubChem recommendation

  - Click Biomolecular Interactions and Pathways, the related target genes collected by PubChem can be seen, saved and downloaded



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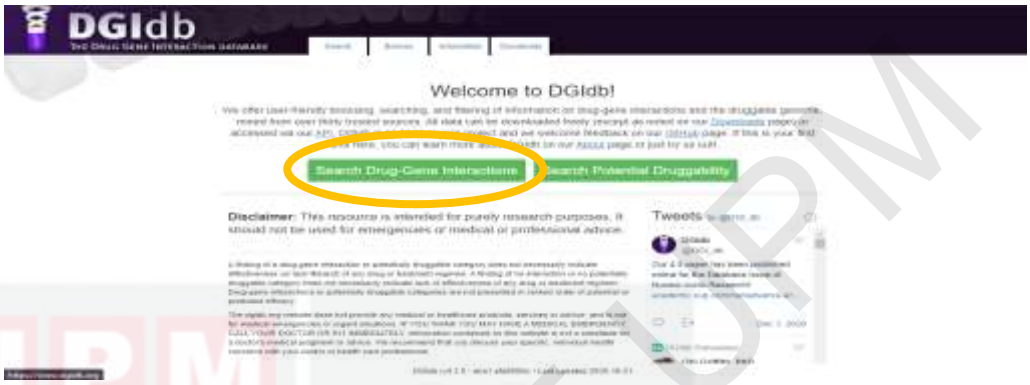
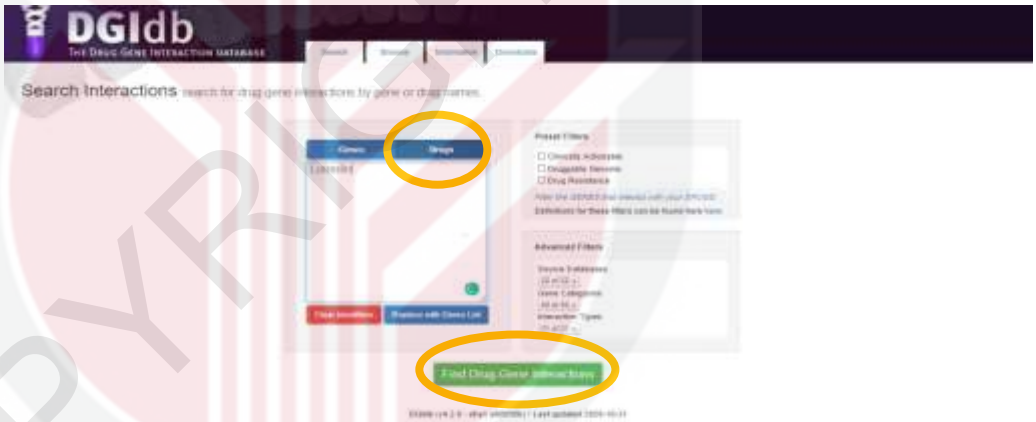
- **DGIdb, this database is more complicated because it cannot be downloaded directly: https://www.dgidb.org/**

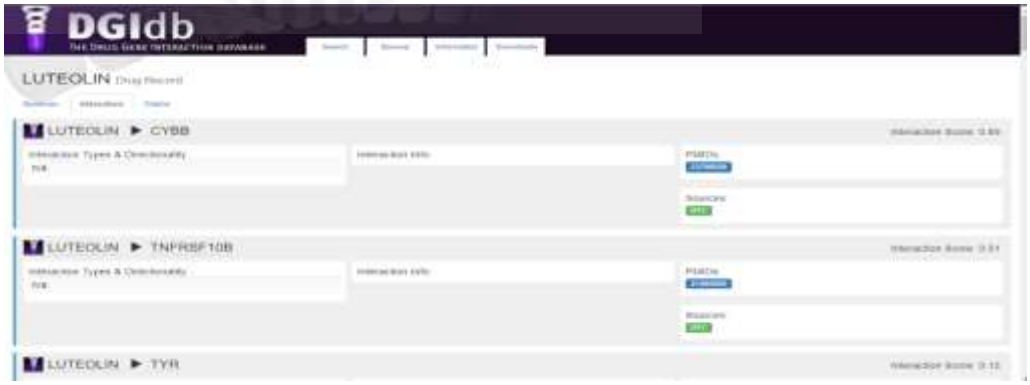
  - Click search drug-gene interactions



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



  - The target genes will be shown



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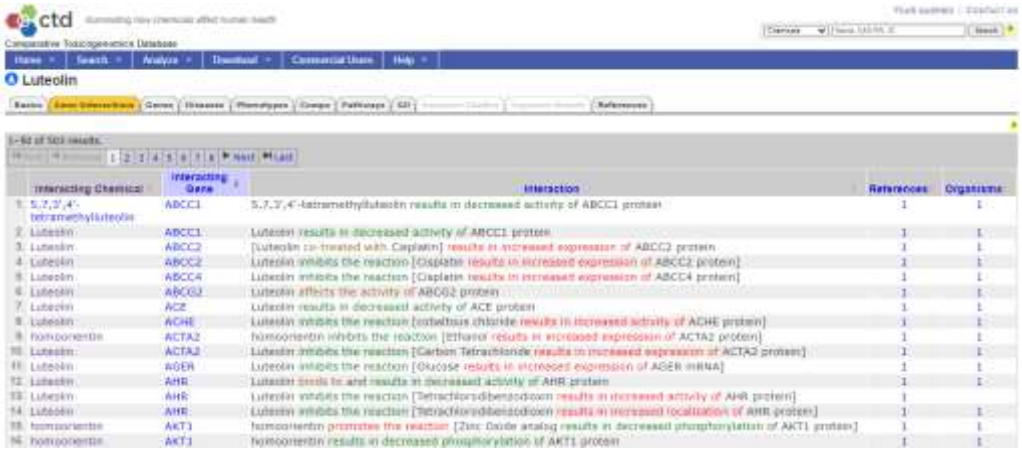
- **Comparative Toxicogenomics Database (CTD): http://ctdbase.org/**

  - Change keyword search to chemical, enter the compound name and click search

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

  - Click gene interaction to see related genes, downloadable at the bottom page



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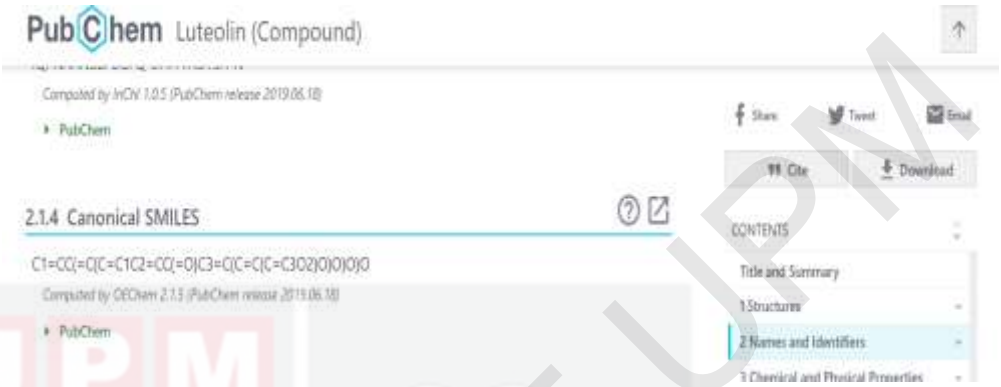
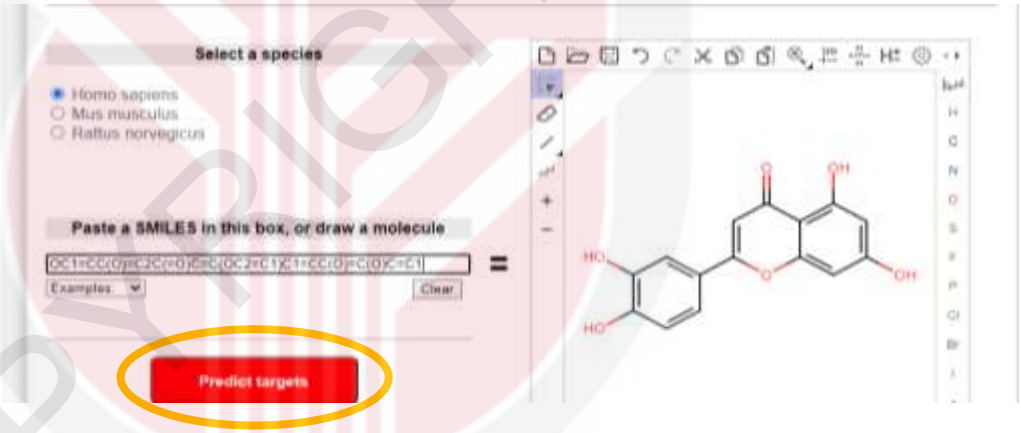
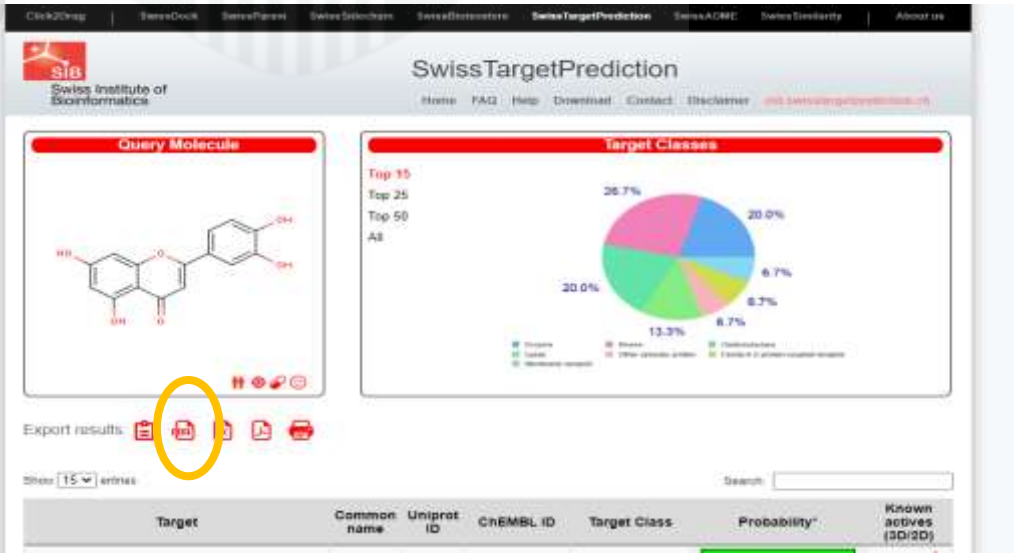
- **Swiss Target Prediction: http://www.swisstargetprediction.ch/**
    - Search for the SMILES of the compound in PubChem: https://pubchem.ncbi.nlm.nih.gov/



    - Select Homo sapiens, copy the SMILES, insert into the respective box shown below and click predict target



    - The result will be shown, and can be export as Excel file



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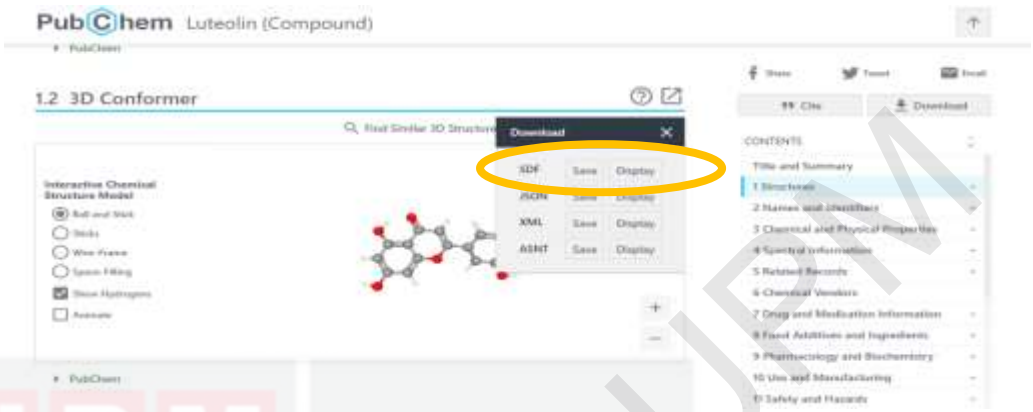
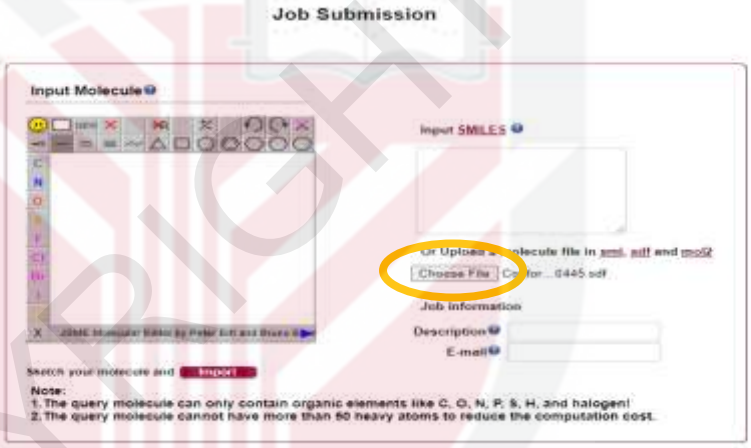
- **Chemmapper: http://www.lilab-ecust.cn/chemmapper/**

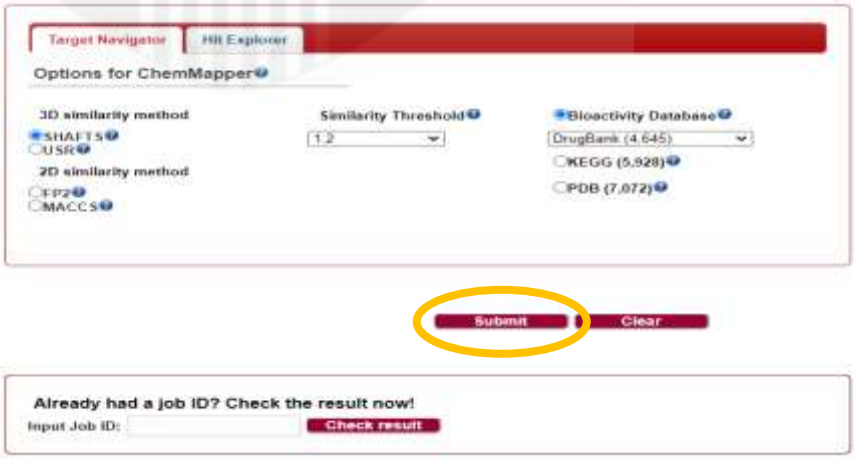
  - Download the sdf file to get 3D-structure from PubChem



  - Upload the sdf file into the database by clicking choose file



  - Click submit



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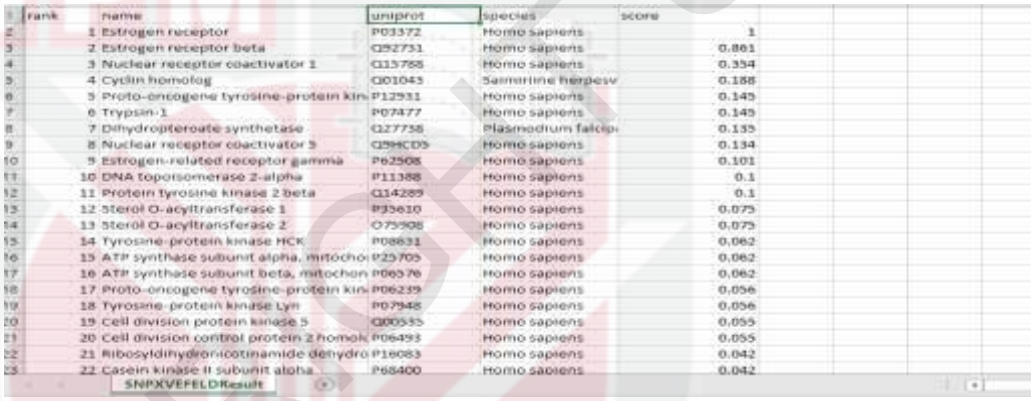
- Then, the result will be given, and can be downloaded

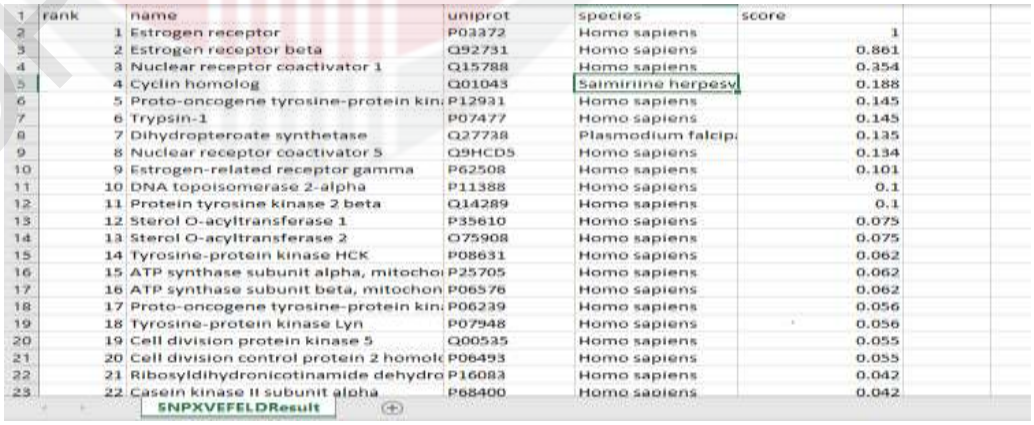
- After got the result, proteins names need to be changed to genes



rank	name	uniprot	species	score
1	Estrogen receptor	P03372	Homo sapiens	1
2	Estrogen receptor beta	Q92731	Homo sapiens	0.861
3	Nuclear receptor coactivator 1	Q15788	Homo sapiens	0.354
4	Cyclin homolog	Q01043	Saimiriine herpesv	0.188
5	Proto-oncogene tyrosine-protein kin	P12931	Homo sapiens	0.145
6	Trypsin-1	P07477	Homo sapiens	0.145
7	Dihydropteroate synthetase	Q27738	Plasmodium falcip	0.135
8	Nuclear receptor coactivator 5	Q9HCD5	Homo sapiens	0.134
9	Estrogen-related receptor gamma	P62508	Homo sapiens	0.101
10	DNA topoisomerase 2-alpha	P11388	Homo sapiens	0.1
11	Protein tyrosine kinase 2 beta	Q14289	Homo sapiens	0.1
12	Sterol O-acyltransferase 1	P35610	Homo sapiens	0.075
13	Sterol O-acyltransferase 2	O75908	Homo sapiens	0.075
14	Tyrosine-protein kinase HCK	P08631	Homo sapiens	0.062
15	ATP synthase subunit alpha, mitocho	P25705	Homo sapiens	0.062
16	ATP synthase subunit beta, mitochon	P06576	Homo sapiens	0.062
17	Proto-oncogene tyrosine-protein kin	P06239	Homo sapiens	0.056
18	Tyrosine-protein kinase Lyn	P07948	Homo sapiens	0.056
19	Cell division protein kinase 5	Q00535	Homo sapiens	0.055
20	Cell division control protein 2 homol	P06493	Homo sapiens	0.055
21	Ribosyldihydronicotinamide dehydro	P16083	Homo sapiens	0.042
22	Casein kinase II subunit alpha	P68400	Homo sapiens	0.042

SNPXVEFELDResult

- Protein not belong to human and score 0 need to be deleted before proceed to UniProt



rank	name	uniprot	species	score
1	Estrogen receptor	P03372	Homo sapiens	1
2	Estrogen receptor beta	Q92731	Homo sapiens	0.861
3	Nuclear receptor coactivator 1	Q15788	Homo sapiens	0.354
4	Cyclin homolog	Q01043	Saimiriine herpesv	0.188
5	Proto-oncogene tyrosine-protein kin	P12931	Homo sapiens	0.145
6	Trypsin-1	P07477	Homo sapiens	0.145
7	Dihydropteroate synthetase	Q27738	Plasmodium falcip	0.135
8	Nuclear receptor coactivator 5	Q9HCD5	Homo sapiens	0.134
9	Estrogen-related receptor gamma	P62508	Homo sapiens	0.101
10	DNA topoisomerase 2-alpha	P11388	Homo sapiens	0.1
11	Protein tyrosine kinase 2 beta	Q14289	Homo sapiens	0.1
12	Sterol O-acyltransferase 1	P35610	Homo sapiens	0.075
13	Sterol O-acyltransferase 2	O75908	Homo sapiens	0.075
14	Tyrosine-protein kinase HCK	P08631	Homo sapiens	0.062
15	ATP synthase subunit alpha, mitocho	P25705	Homo sapiens	0.062
16	ATP synthase subunit beta, mitochon	P06576	Homo sapiens	0.062
17	Proto-oncogene tyrosine-protein kin	P06239	Homo sapiens	0.056
18	Tyrosine-protein kinase Lyn	P07948	Homo sapiens	0.056
19	Cell division protein kinase 5	Q00535	Homo sapiens	0.055
20	Cell division control protein 2 homol	P06493	Homo sapiens	0.055
21	Ribosyldihydronicotinamide dehydro	P16083	Homo sapiens	0.042
22	Casein kinase II subunit alpha	P68400	Homo sapiens	0.042

SNPXVEFELDResult

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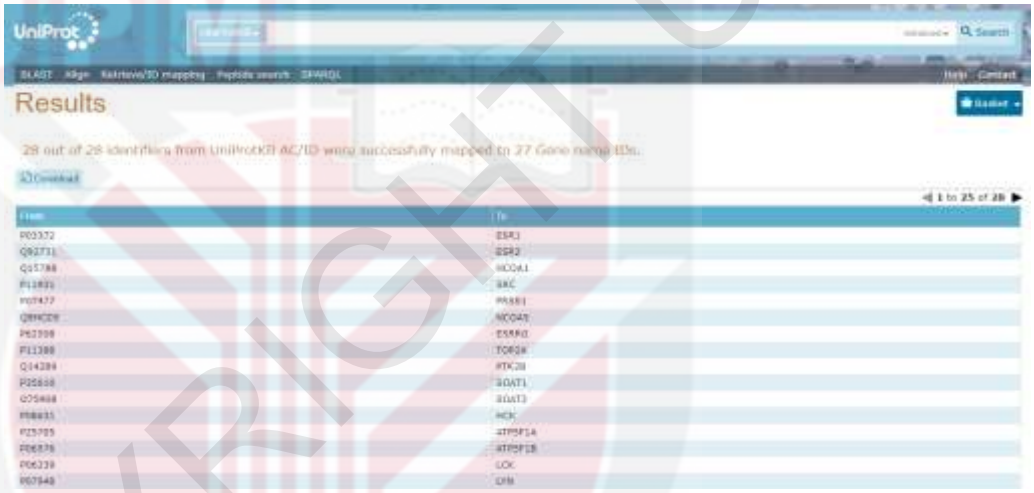
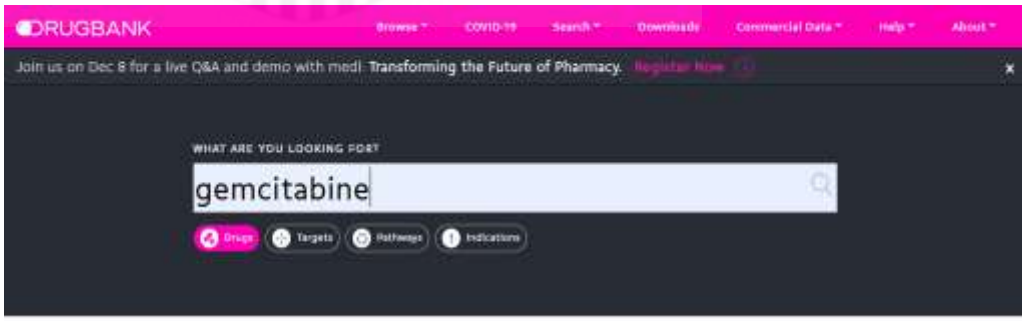
- Then go to UniProt https://www.uniprot.org/uploadlists/. Insert the identifiers & click submit

- The result will be shown



2. Target Genes of Gemcitabine

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



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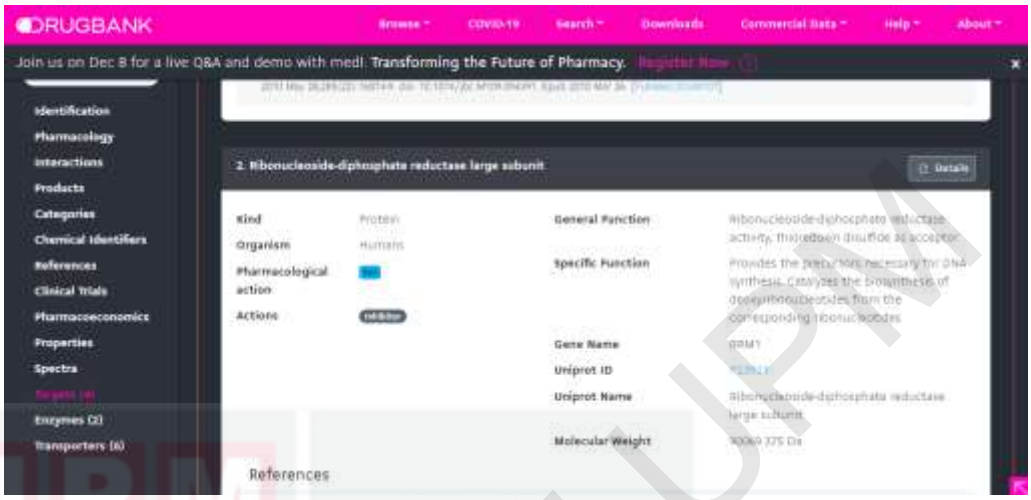
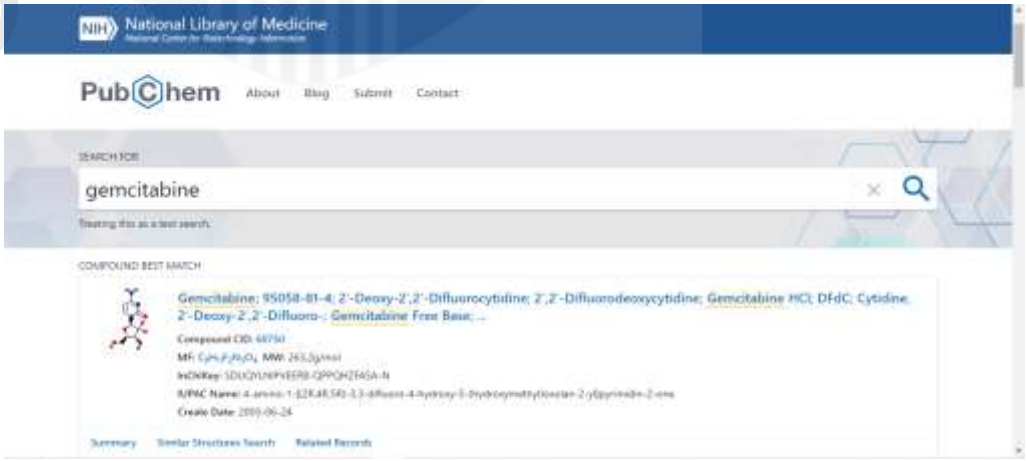
- Generally, the target gene will be given after SPECTRA



- **PubChem:** https://pubchem.ncbi.nlm.nih.gov/

- Insert the drug name

- Choose PubChem recommendation



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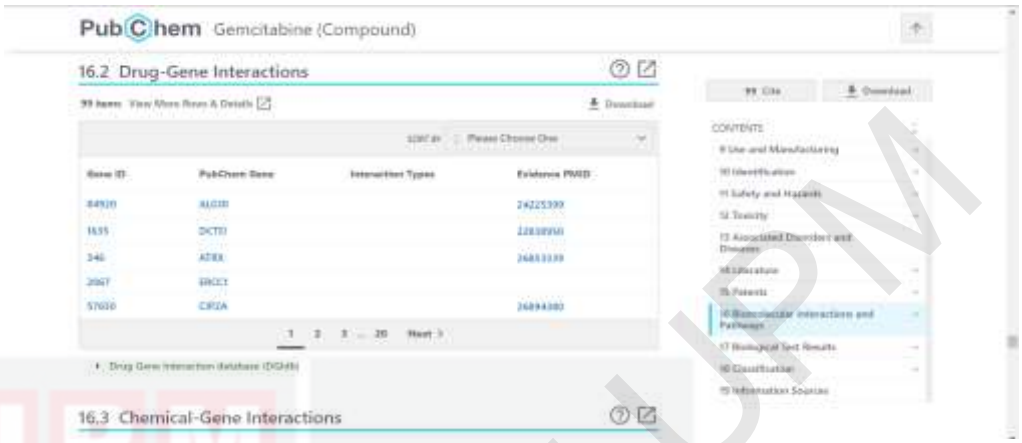
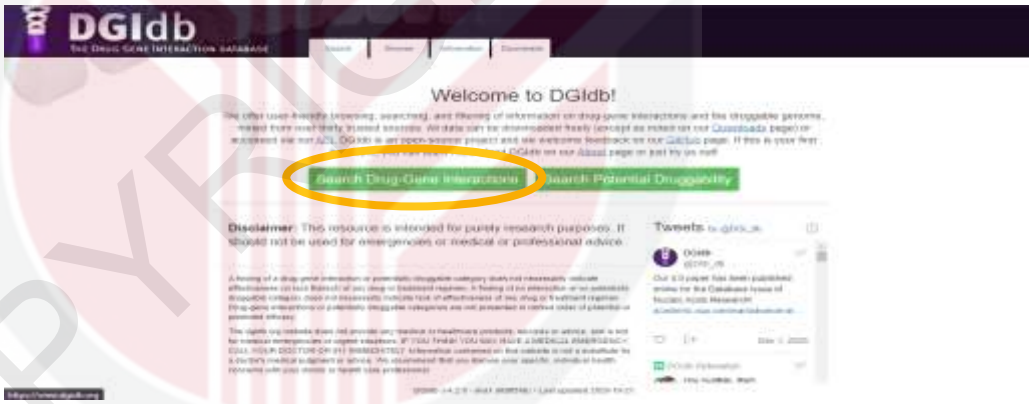
- Click Biomolecular Interactions and Pathways, the related target genes collected by PubChem can be seen, saved and downloaded

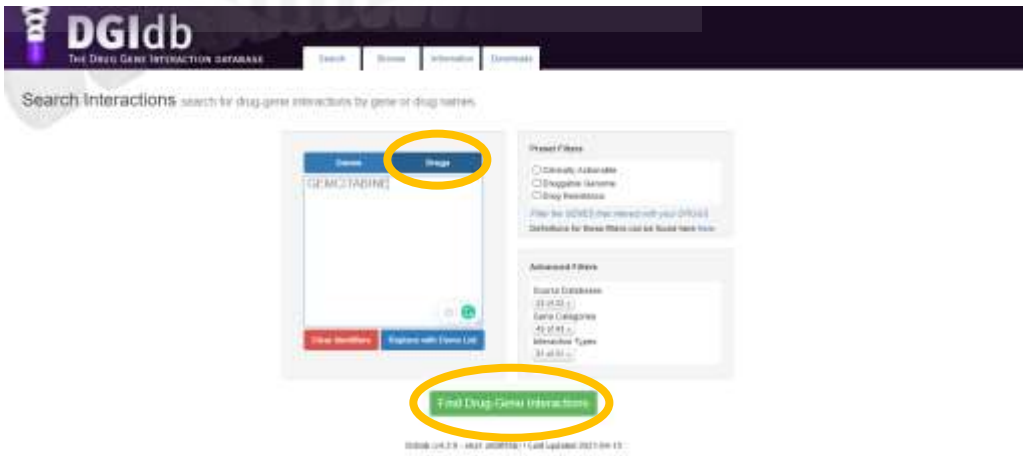


- **DGIdb, this database is more complicated because it cannot be downloaded directly: https://www.dgidb.org/**

- Click search drug-gene interactions



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



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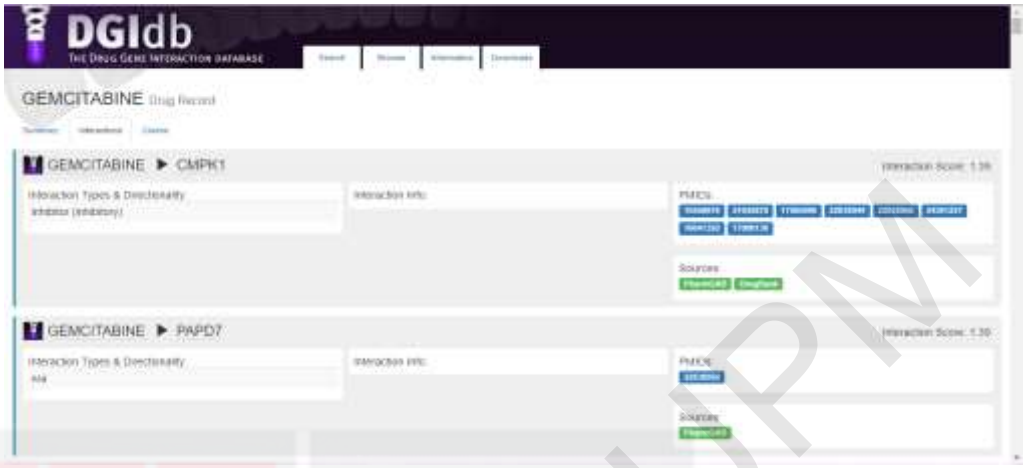
- The target genes will be shown



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

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

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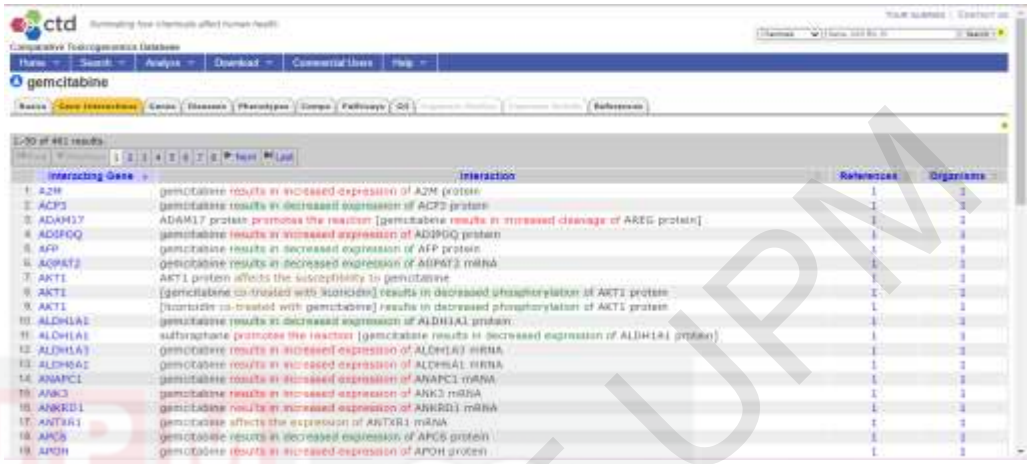
- Click gene interaction to see related genes, downloadable at the bottom page



3. Target Genes of Pancreatic Cancer

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

  - Click gene

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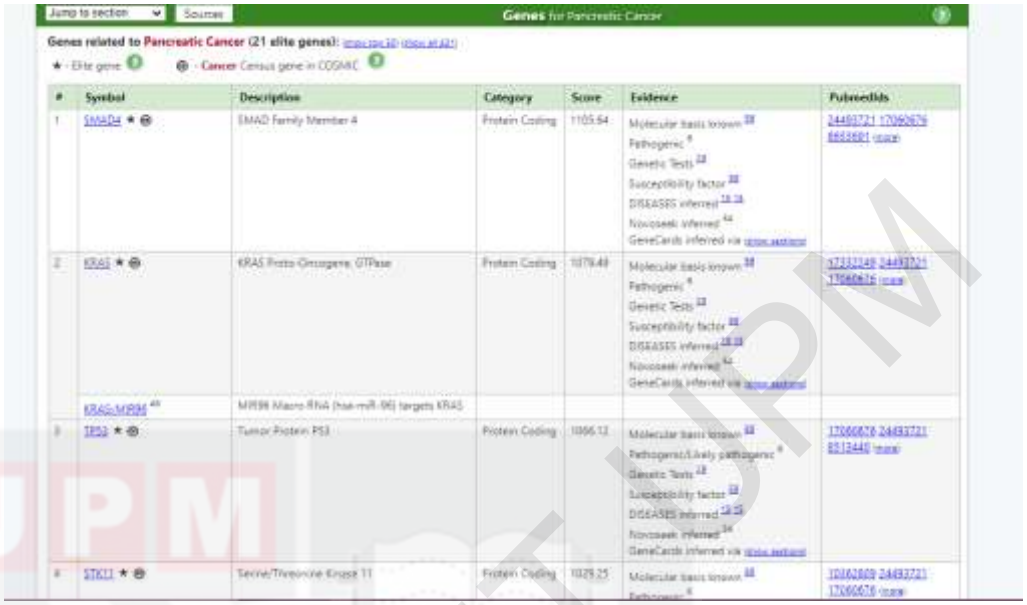
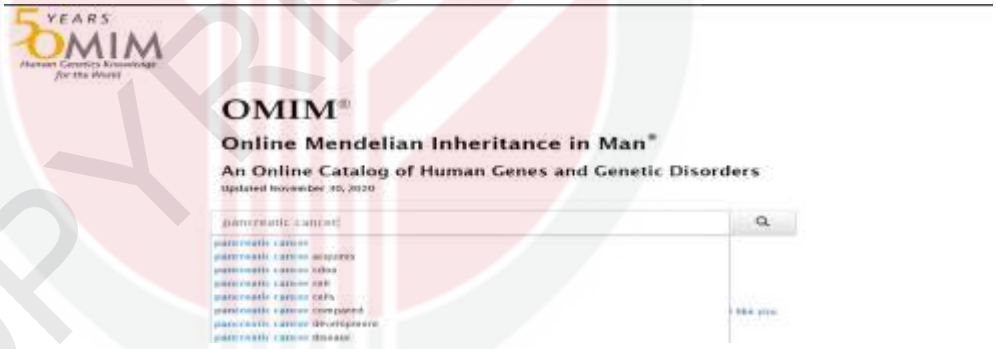
- The result will be shown

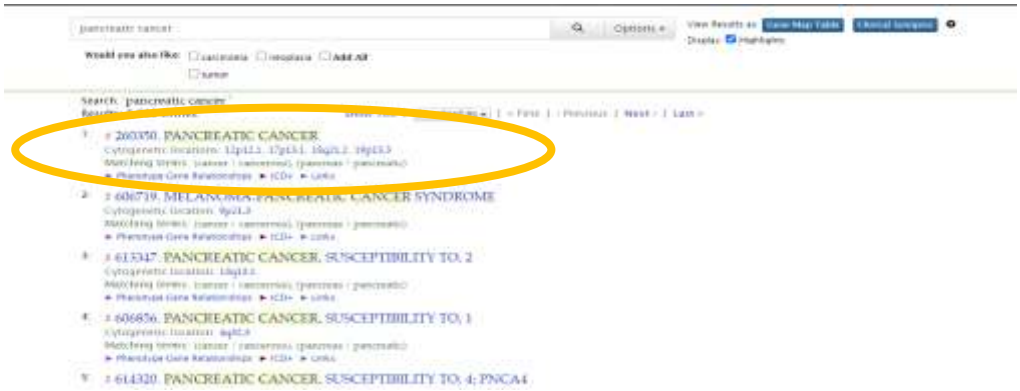


- **Online Mendelian Inheritance in Man (OMIM) database: https://www.omim.org/**

  - Enter the disease name: Pancreatic cancer and click search



  - Choose the first one



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- The result of pancreatic cancer-related gene will be shown

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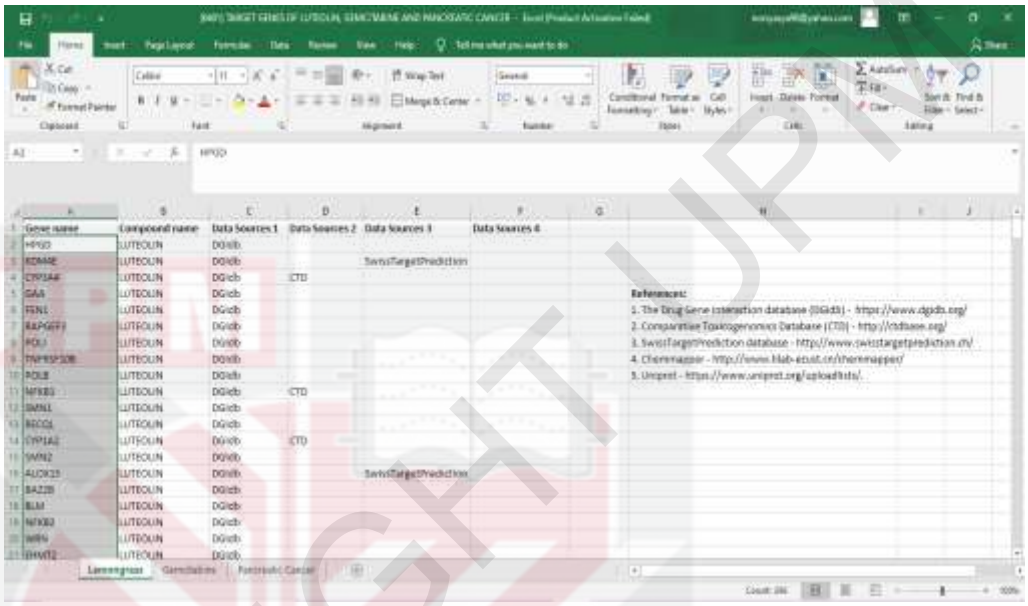
APPENDIX IV

Screening Potential Targets of Lemongrass and Gemcitabine

- Copy all the genes listed in the excel file



- Go to http://bioinformatics.psb.ugent.be/webtools/Venn/ and scroll down to see the input section

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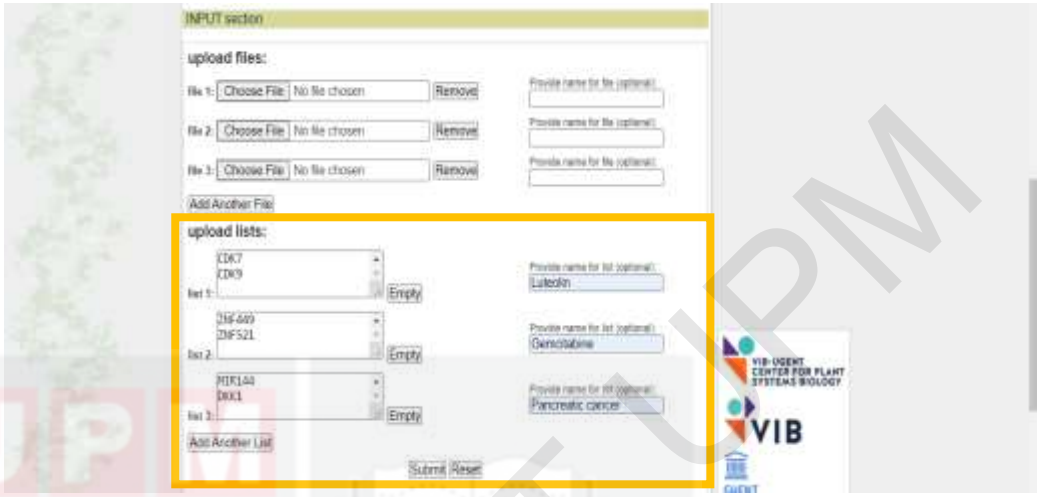
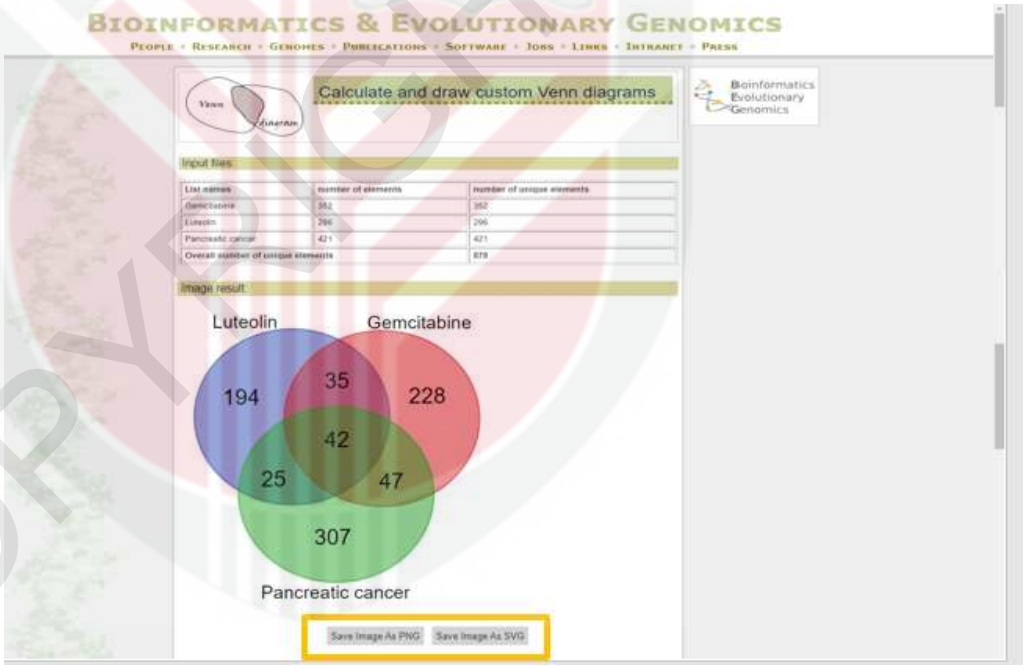
- Paste all the genes for the compound, drug and disease in the respective box, provide the name for each of them and click submit



- The image and text results will be shown and can be saved at the bottom



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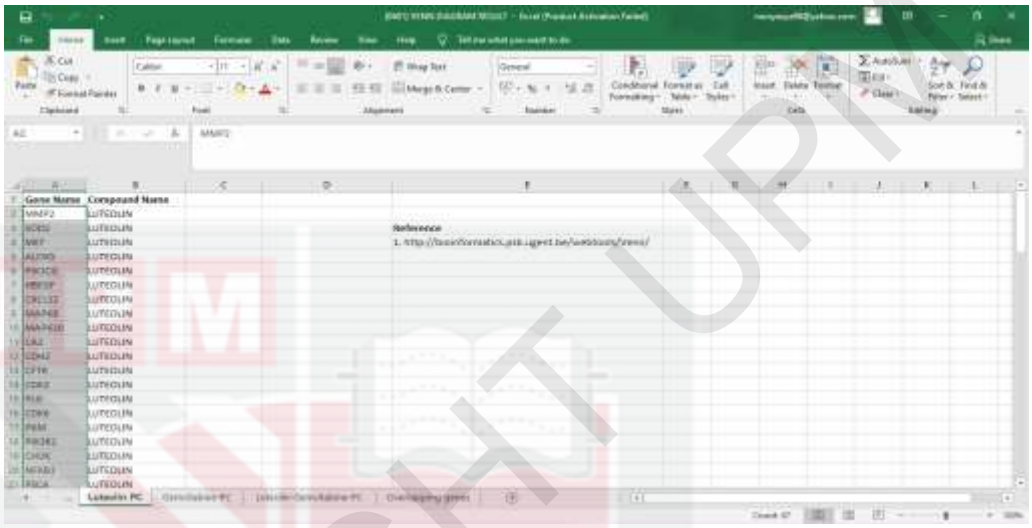
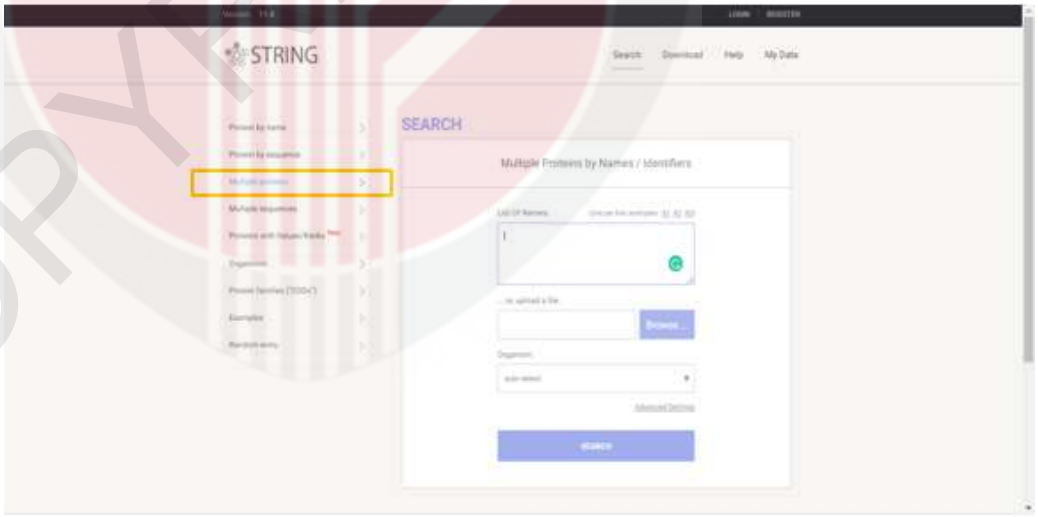
APPENDIX V

Protein-Protein Interaction (PPI) Network

- Copy the overlapping target genes from the Excel file



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



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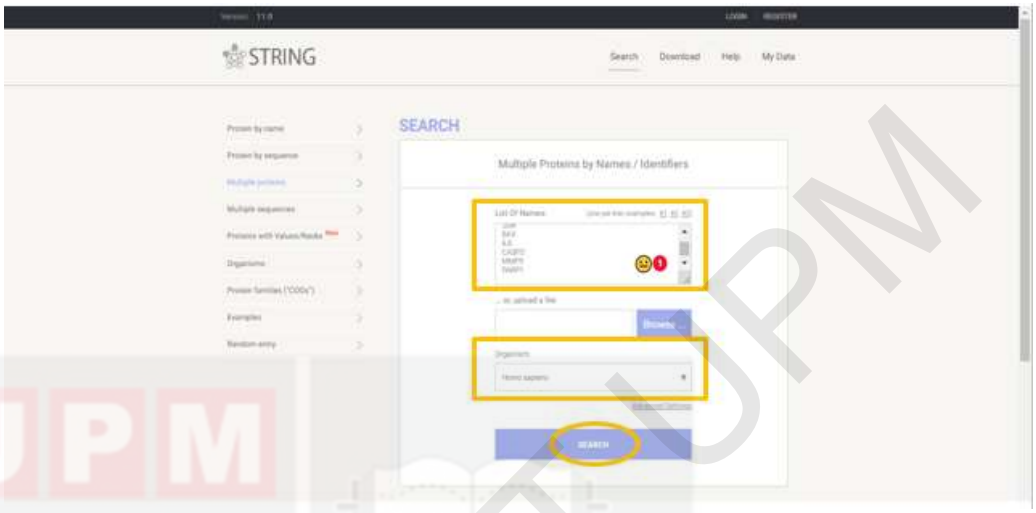
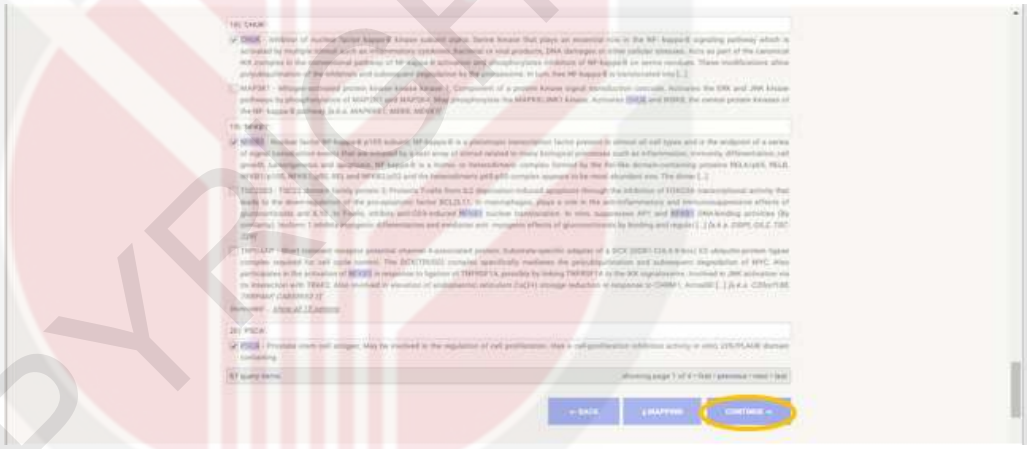
- Paste the overlapping genes, choose organism as Homo sapiens and click search



- Scroll down and click continue



- The result will be appeared

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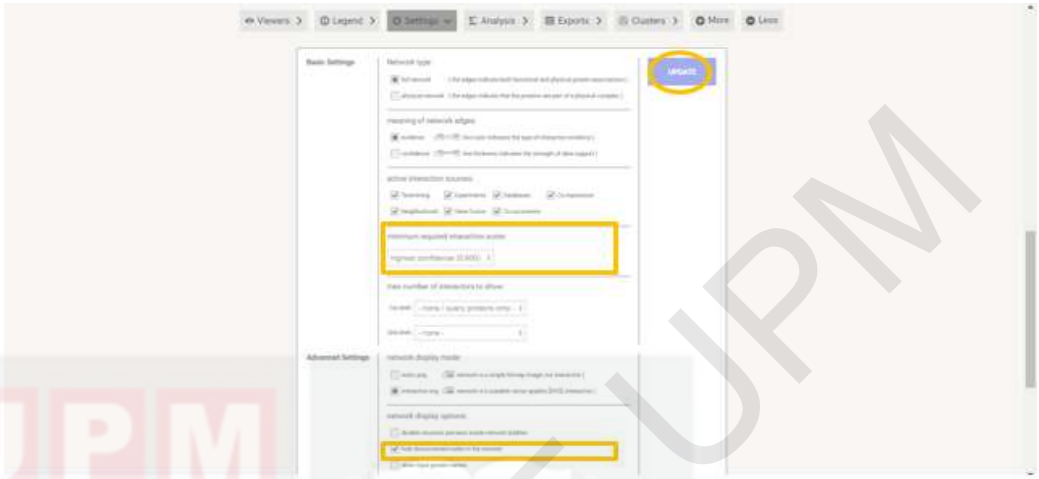
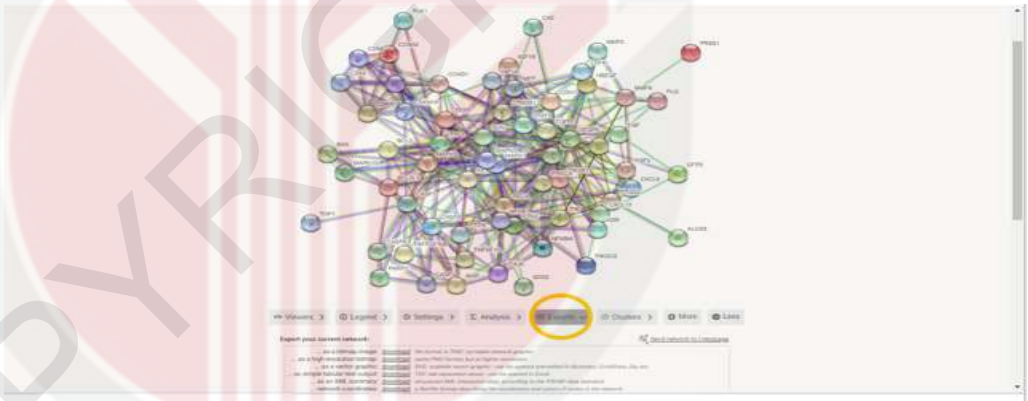
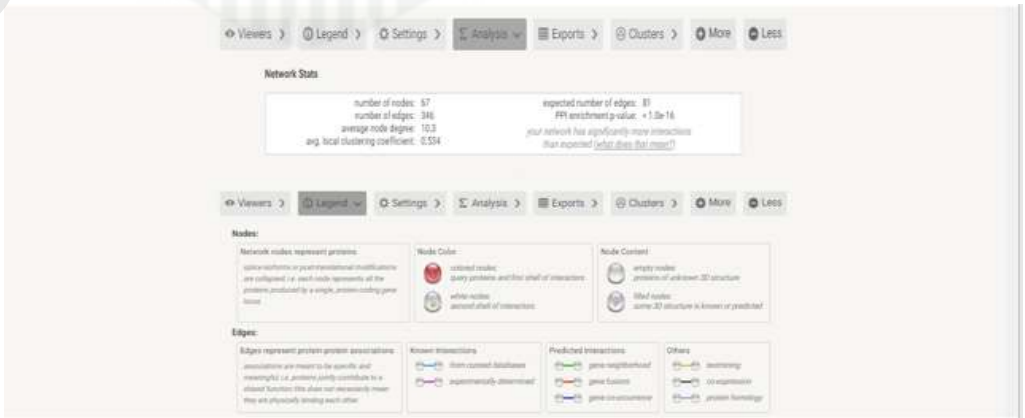
- To get a nice result, go to setting, choose minimum required score as 0.900, hide disconnected nodes in the network and click update



- The updated result will be appeared, and can be downloaded by clicking export



- The result of the analysis and the legend



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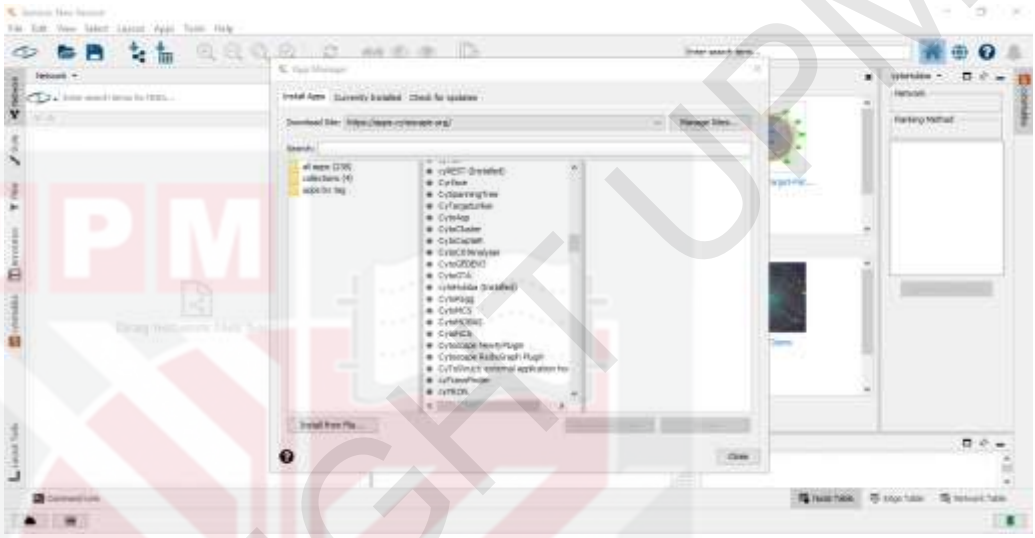
APPENDIX VI

Identification of Hub Genes

- Open Cytoscape and go to app > app manager to install cytoHubba to the software



- Import string_interactions file into the software by clicking file > import > network from file

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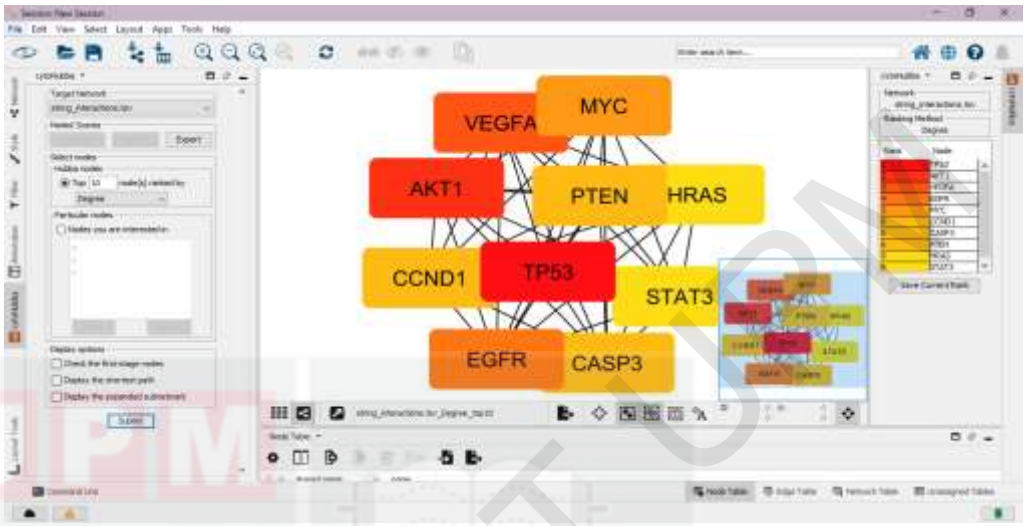
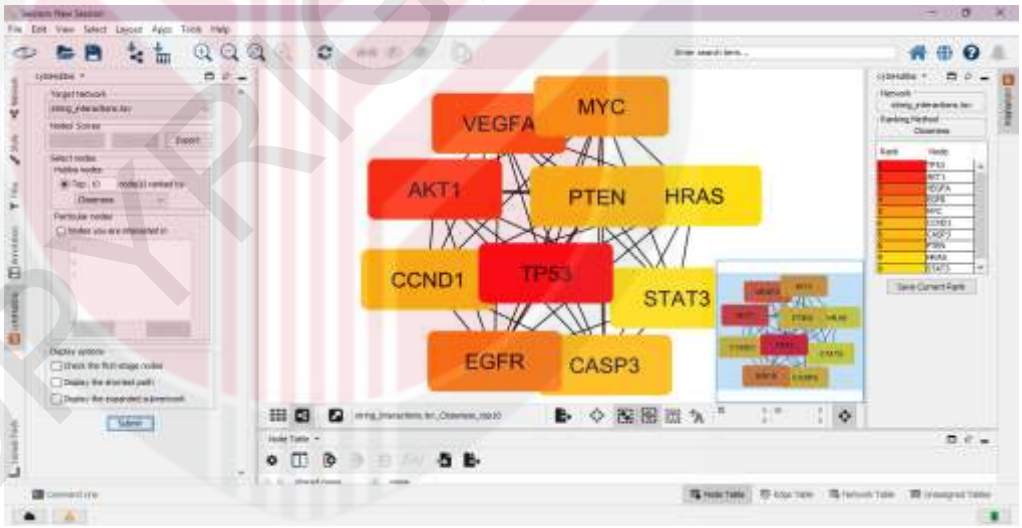
- Go to cytoHubba > calculate > select nodes as “top 10” > ranked by degree. The result will come out next to it



- Go to cytoHubba > calculate > select nodes as “top 10” > ranked by closeness. The result will come out next to it



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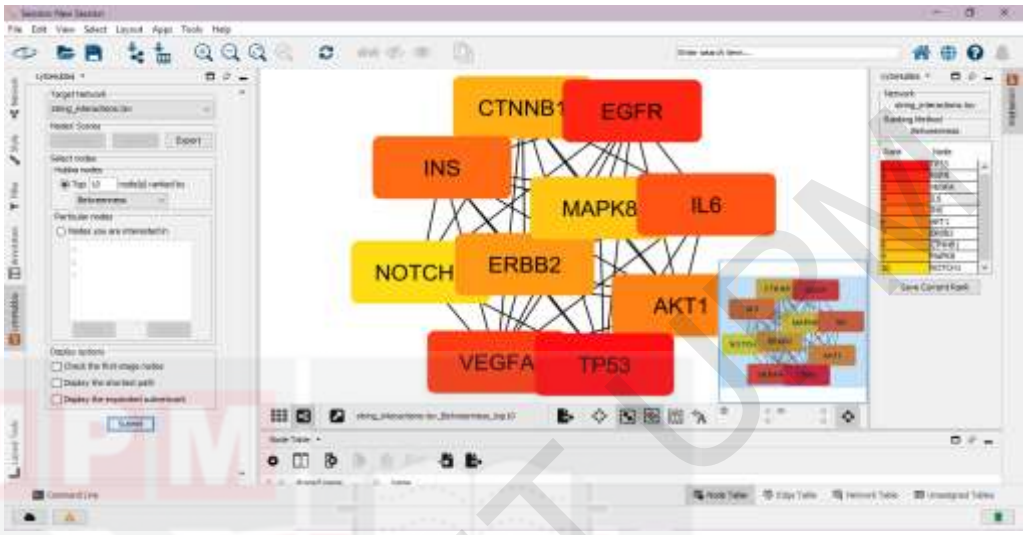
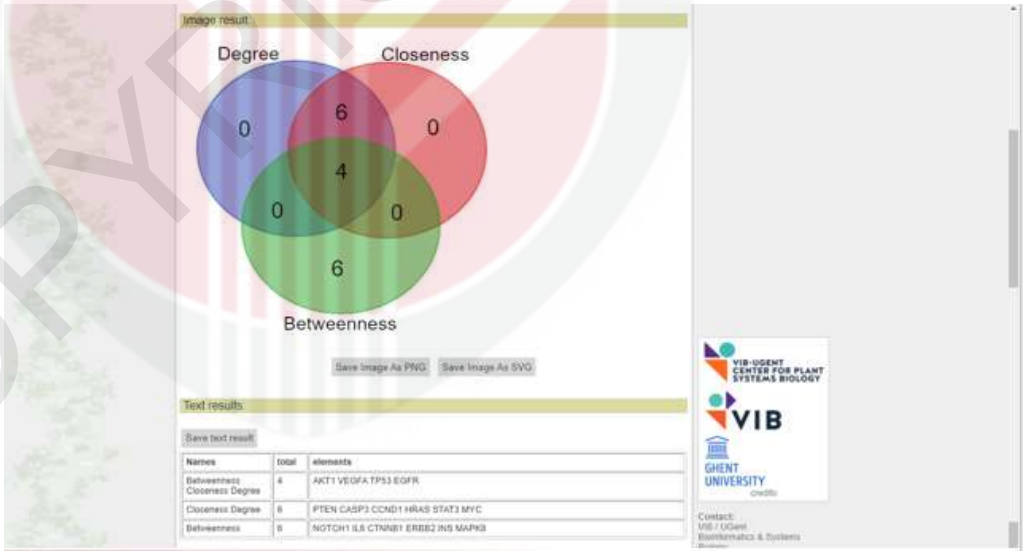
- Go to cytoHubba > calculate > select nodes as “top 10” > ranked by betweenness. The result will come out next to it



- Get the intersection by putting all the genes for three calculation method into Bioinformatics and Evolutionary Genomics http://bioinformatics.psb.ugent.be/webtools/Venn/



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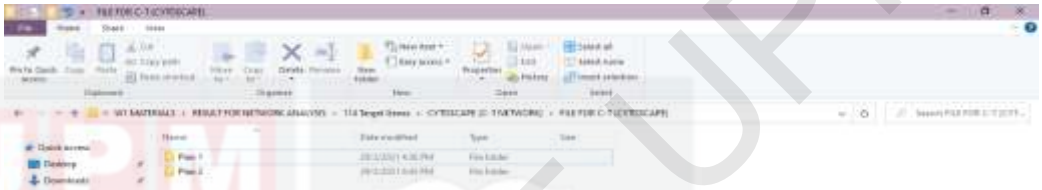
APPENDIX VII

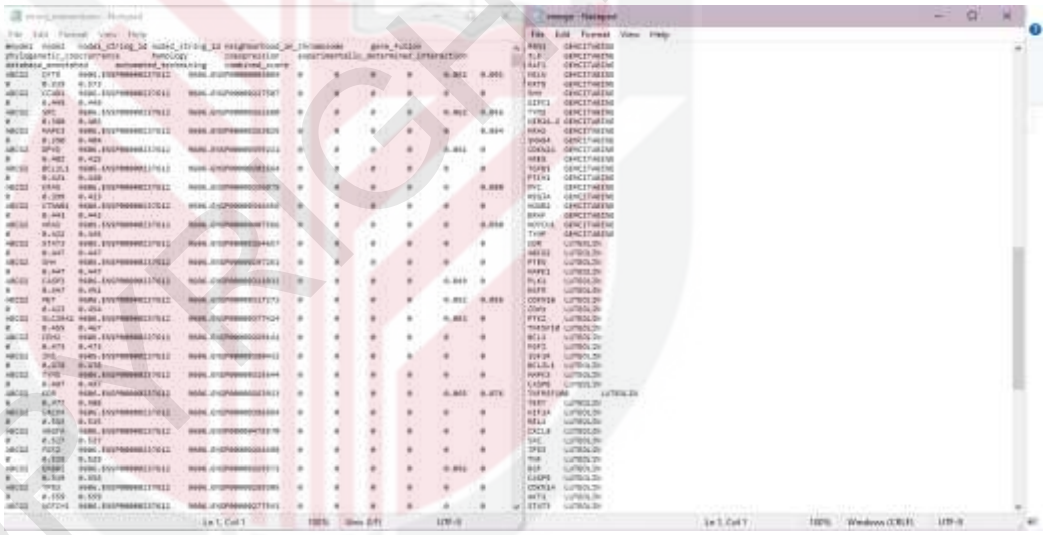
Network Construction

1. Compound-Target Network

- Before constructing the compound-target network, 2 plan files consist of 5 individual files need to be prepared first.



i) Plan 1 files: string_interaction and merge file



ii) geneList, network and node file in txt format

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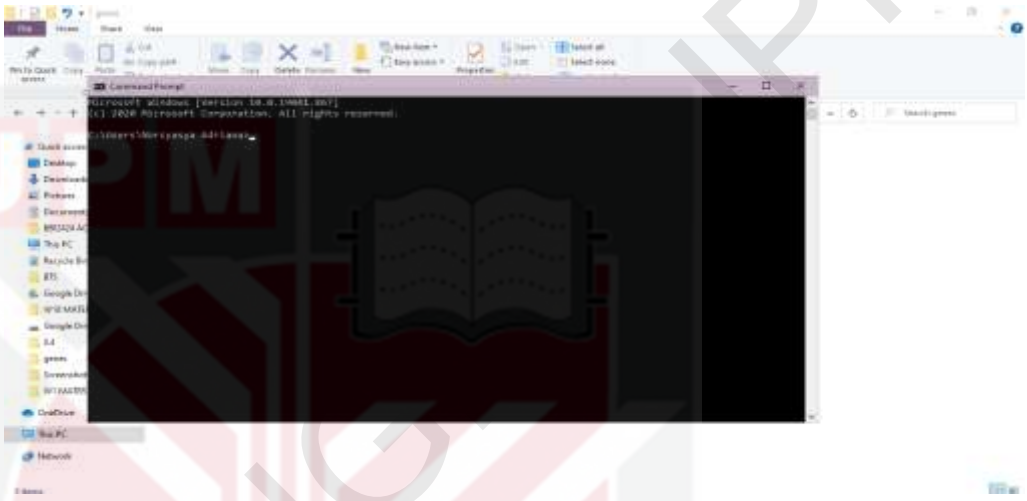
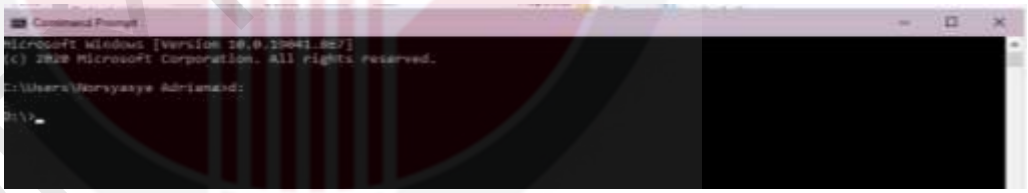
Step 1: Put plan 1 and perl file in gene file

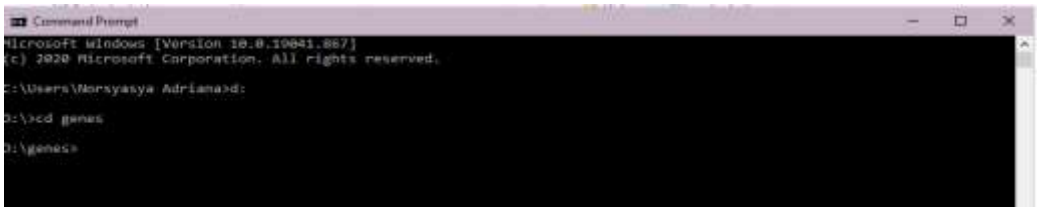
Step 2: Open Command Prompt



Step 3: Type d: and press enter



Step 4: Type cd genes and press enter



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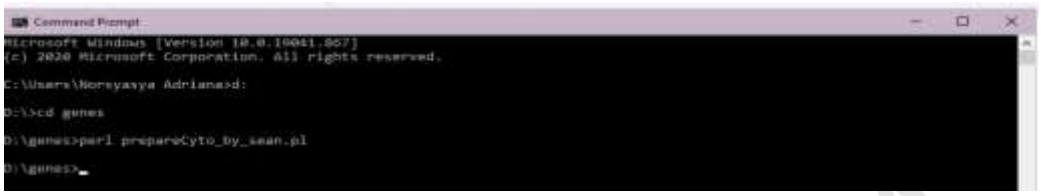
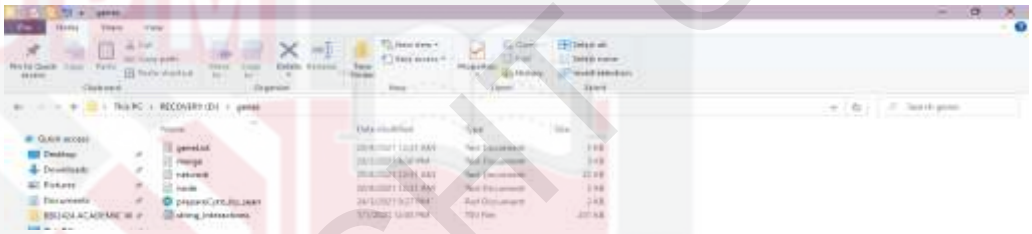
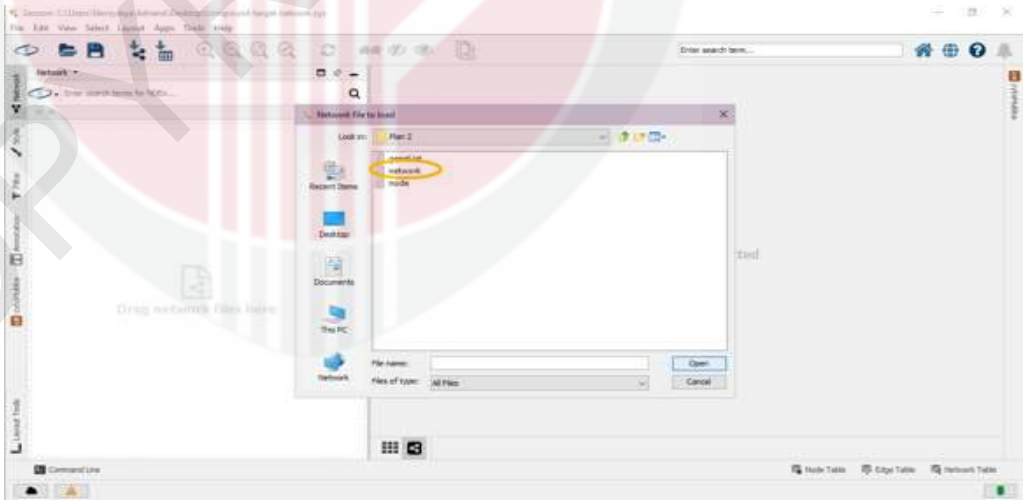
Step 5: Type perl prepareCyto_by_sean.pl and press enter



Step 6: geneList, network and node txt files will automatically be appeared and then put in one folder named as “Plan 2” file. Check also the result and add the missing data



- Open Cytoscape and import the prepared file (network) into the software by clicking file > import > network from file



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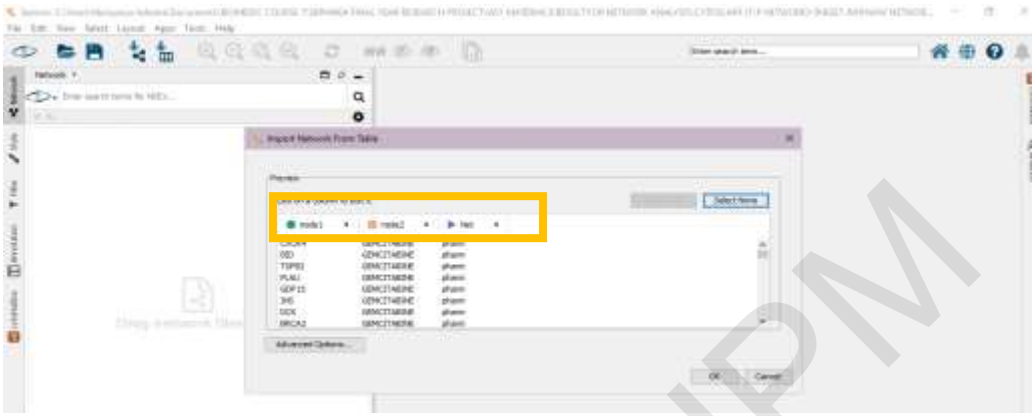
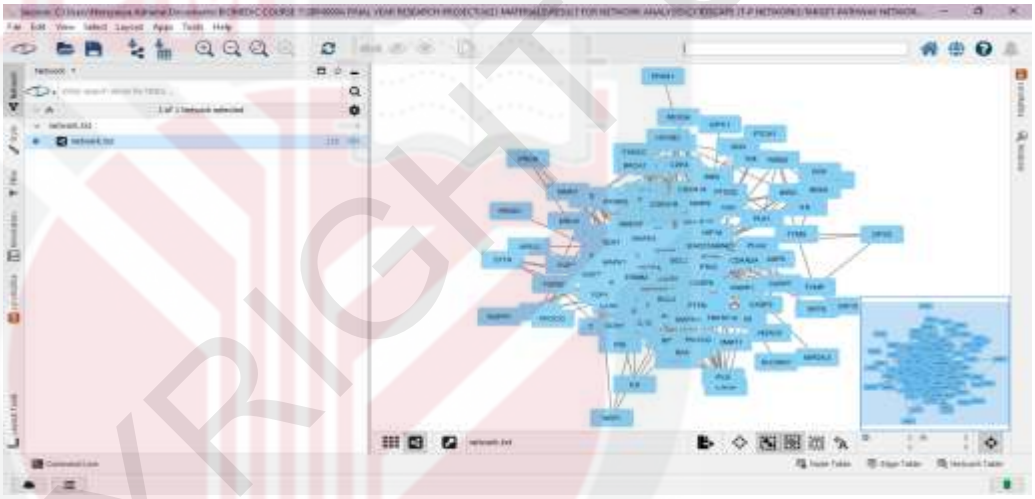
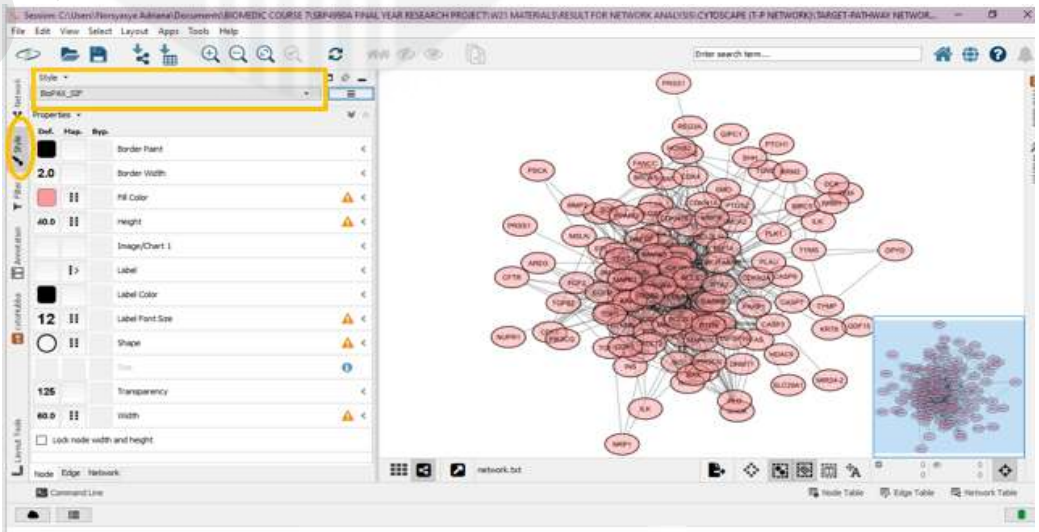
- Edit each column like in the figure below and click OK



- The result will be appeared



- Can change the network according to our preference by clicking the style button



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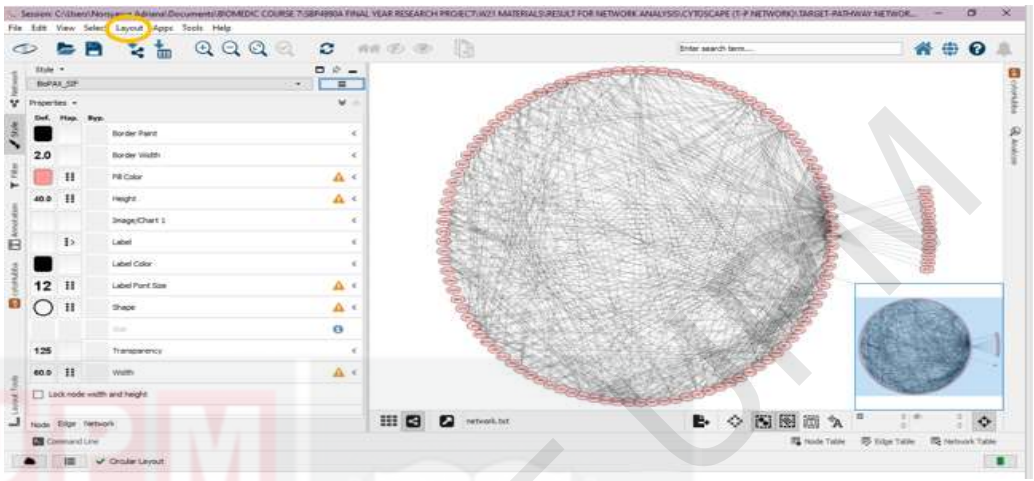
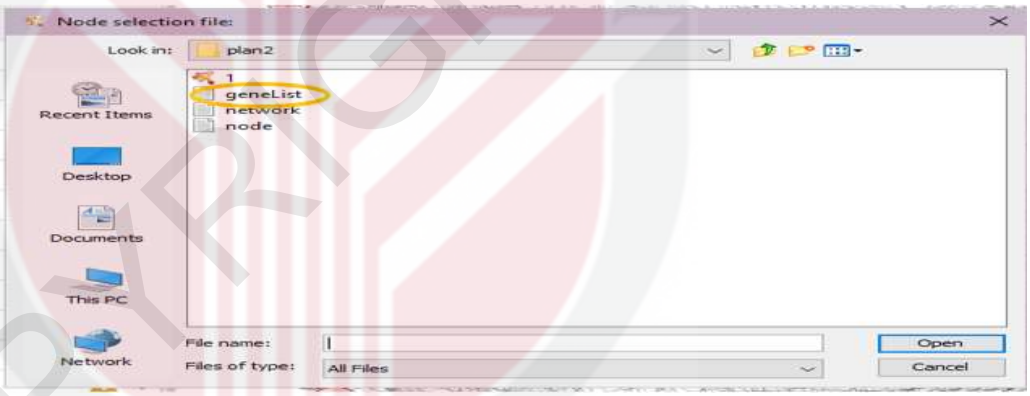
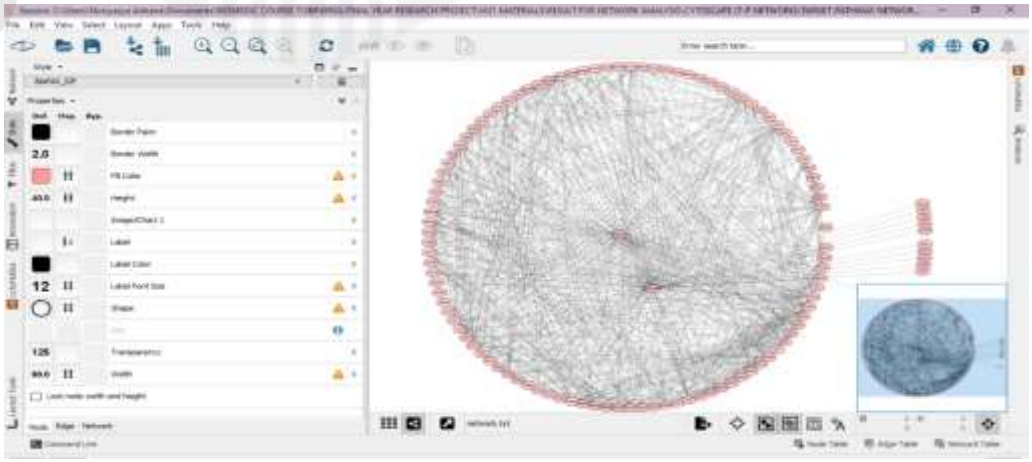
- Can also change the layout. Like the figure below is layout > circular layout



- To put the compound in the middle, go to select > nodes > from ID List File and choose geneList file and click open



- Then it will show like this after move the compound to the middle



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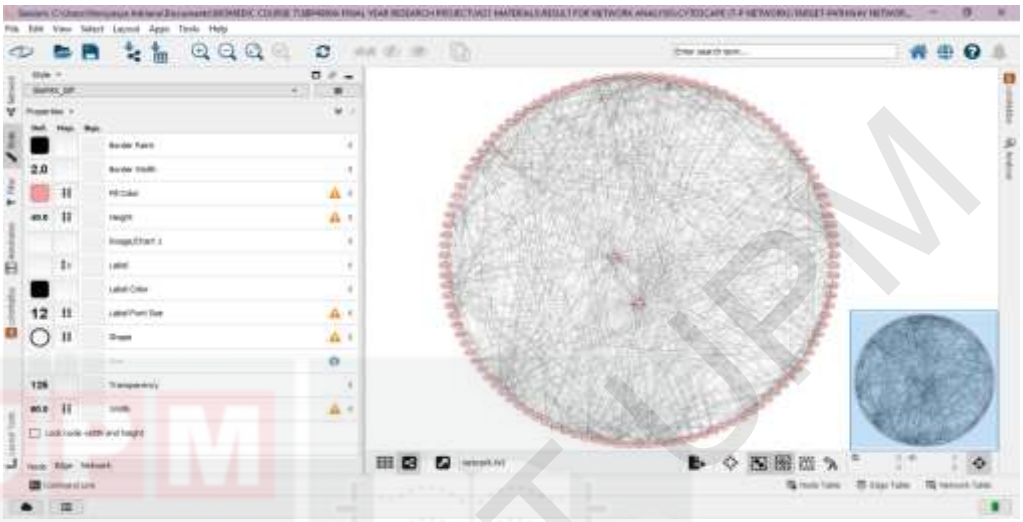
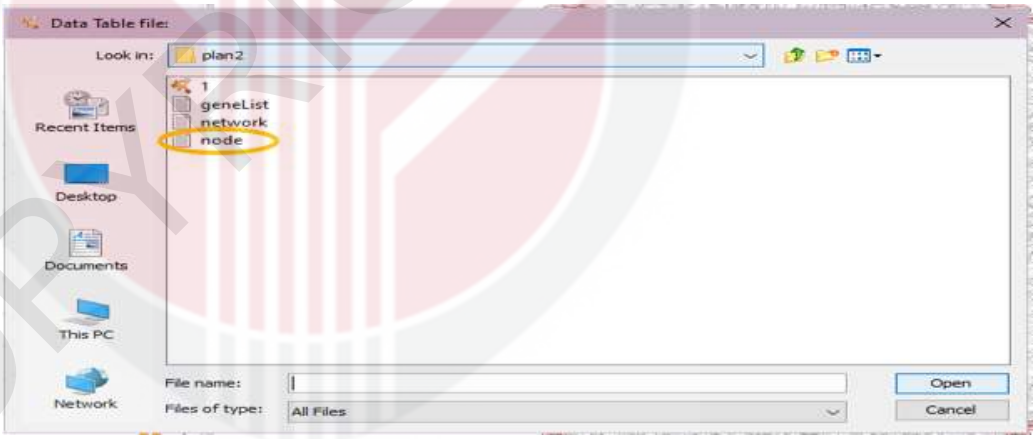
- To arrange to complete circle, go to layout > attribute circle layout > selected nodes only > names and the result will give like this



- Before proceed with editing the nodes, we need to import the nodes file to provide information for the Cytoscape by clicking file > import > table from file



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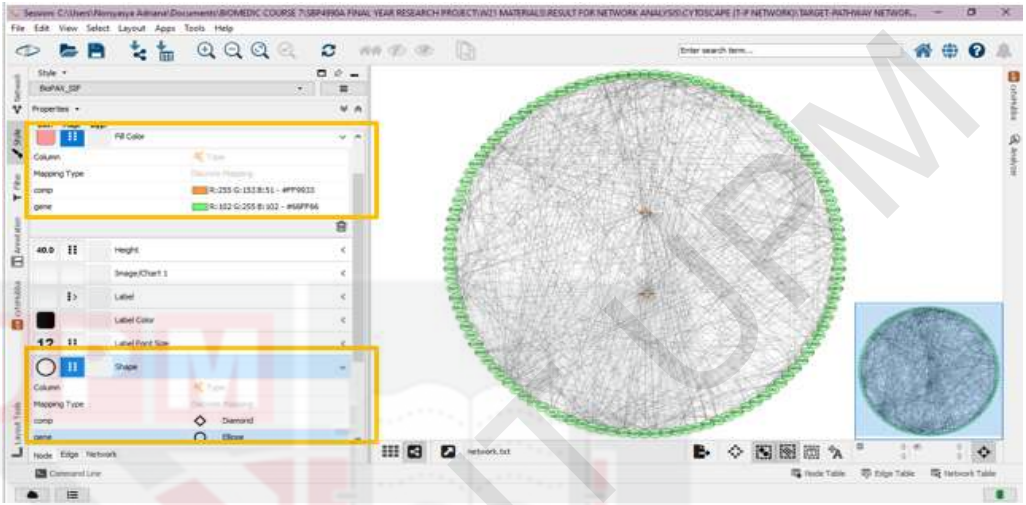
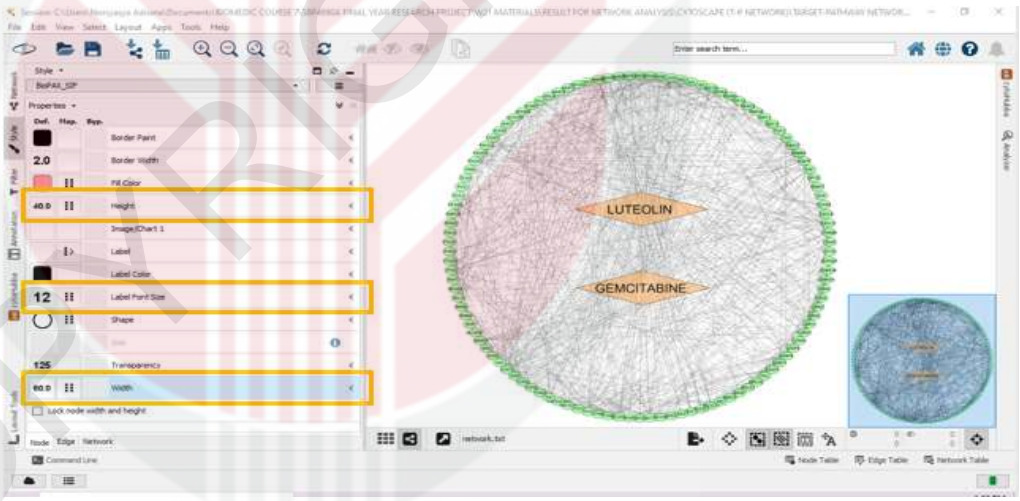
- Choose different fill color and shape for compounds and genes to differentiate between them by editing the fill color and shape like shown in the yellow boxes below here



- Can also change the height, font size and width of the nodes



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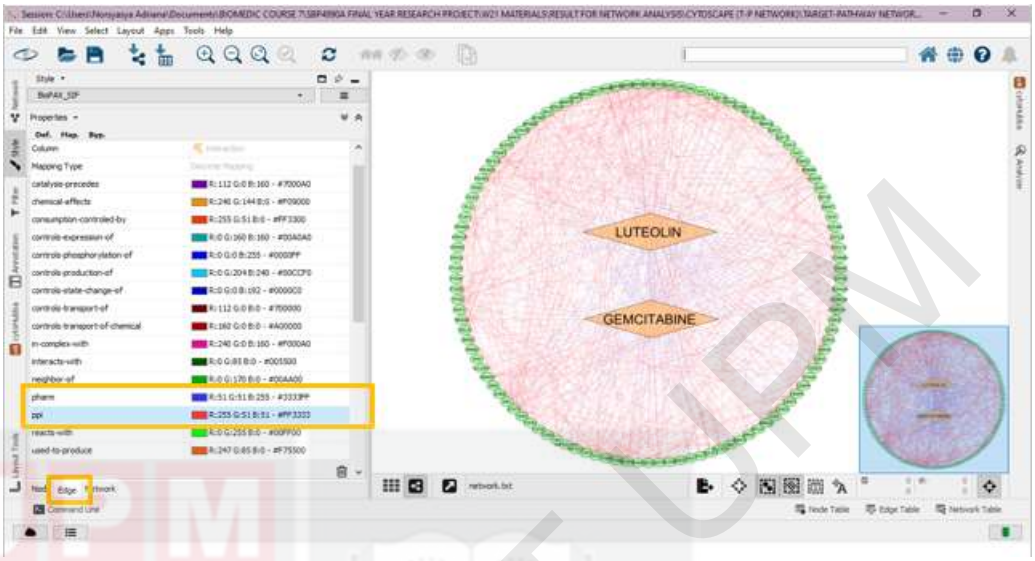
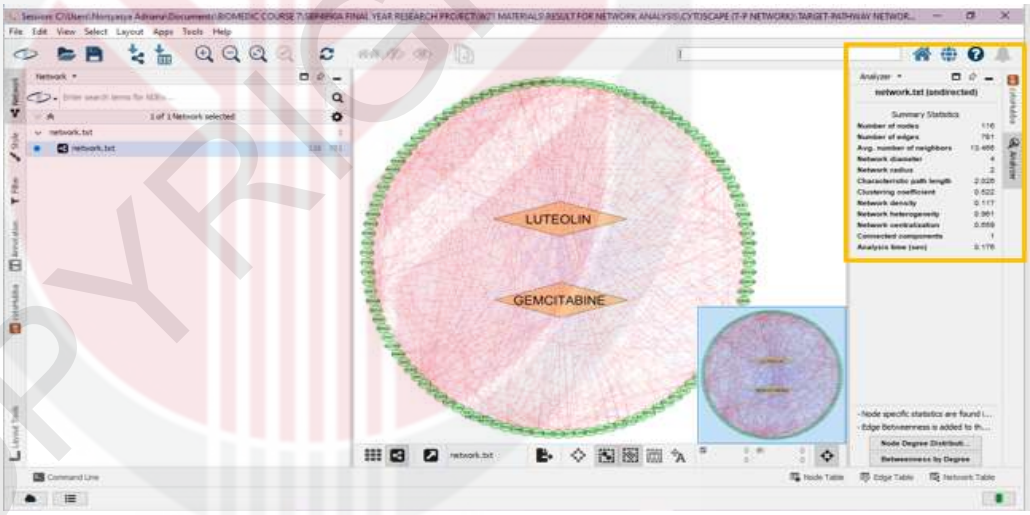
- Click edge to change the color



- To see the result of the analysis, click tool > analyze network. The result will be appeared on the right side



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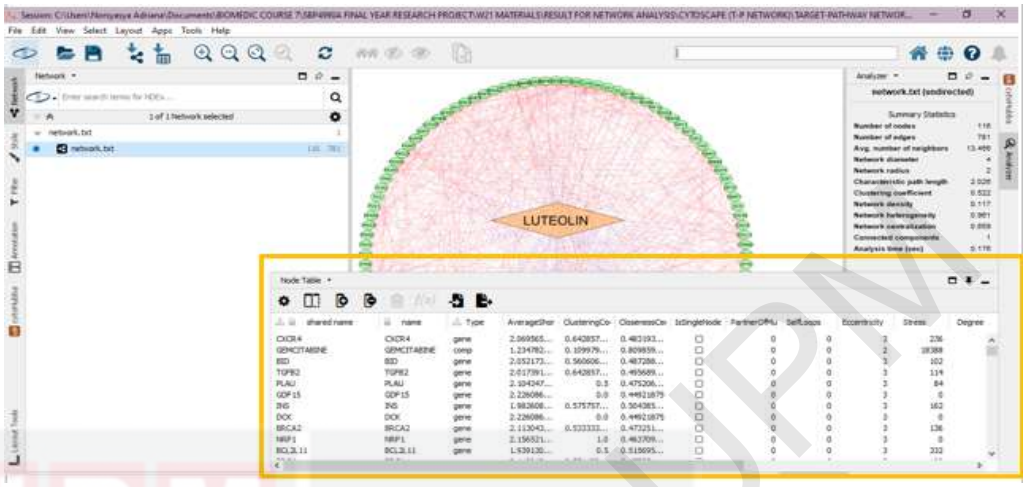
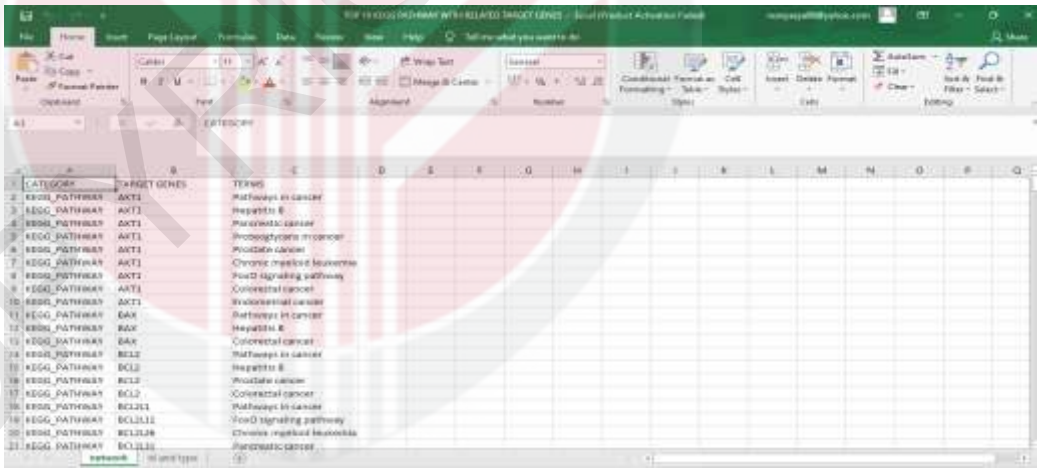
- The node information can also be downloaded in Excel format



2. Target-Pathway Network

- Before constructing the target-pathway network, 3 files need to be prepared first

  i) Top 10 KEGG pathway in excel format



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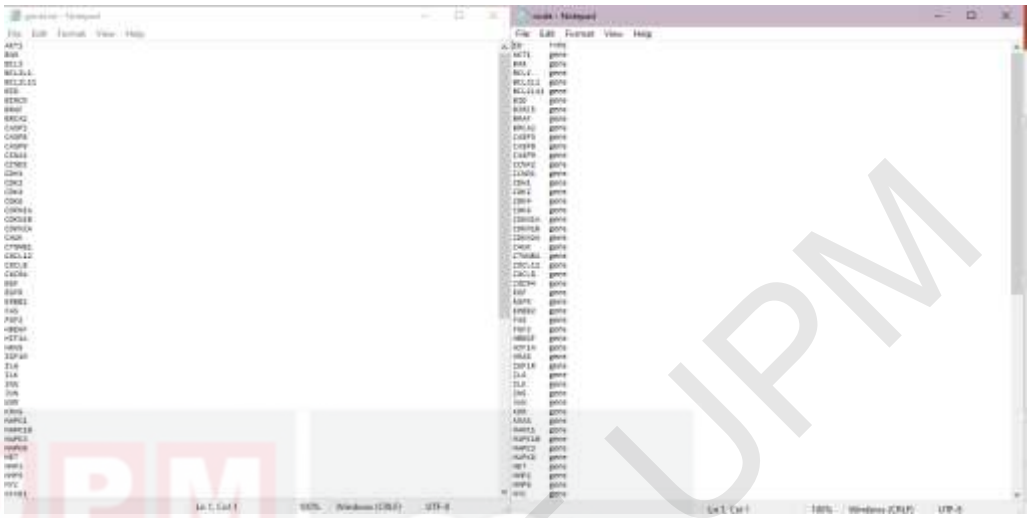
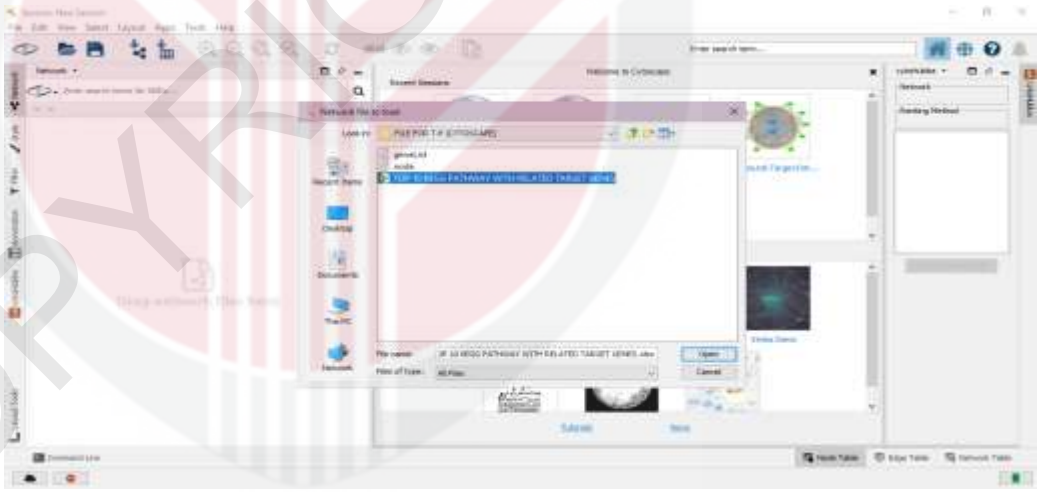
ii) geneList and node files in txt format



- Open Cytoscape software to start constructing the target-pathway network and import the prepared file into the software by clicking file > import > network from file



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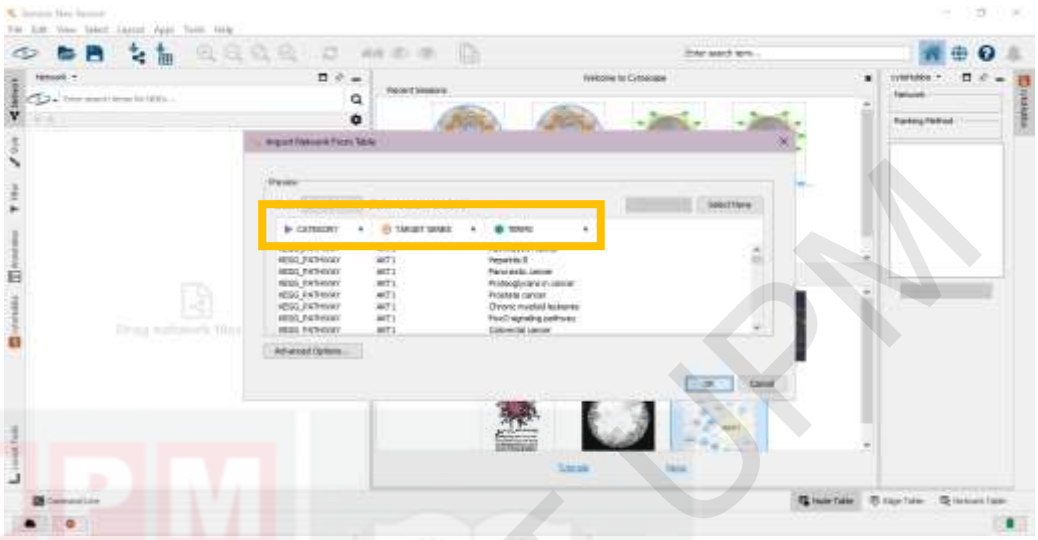
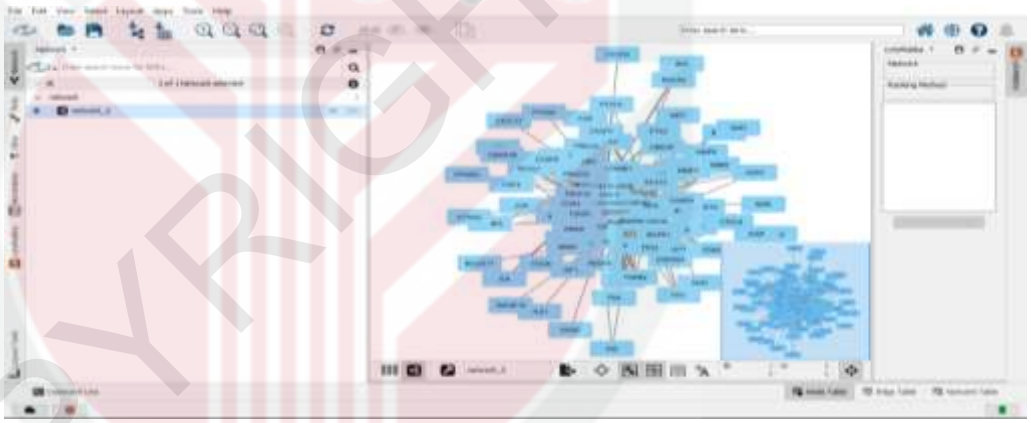
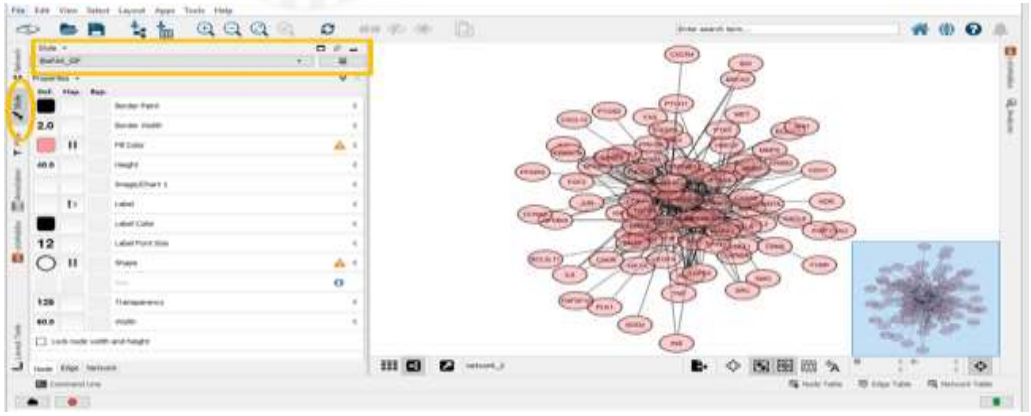
- Edit each column like in the figure below and click OK



- The result will be appeared



- Can change the network according to our preference by clicking the style button



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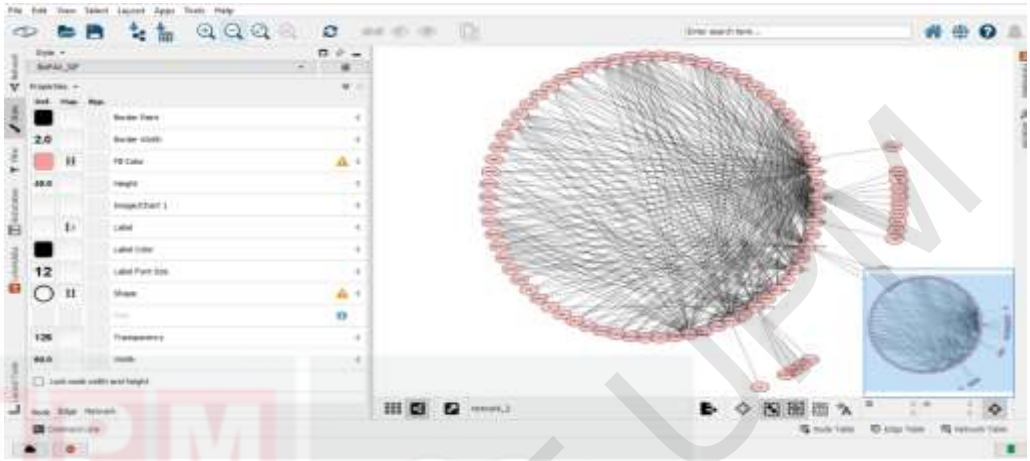
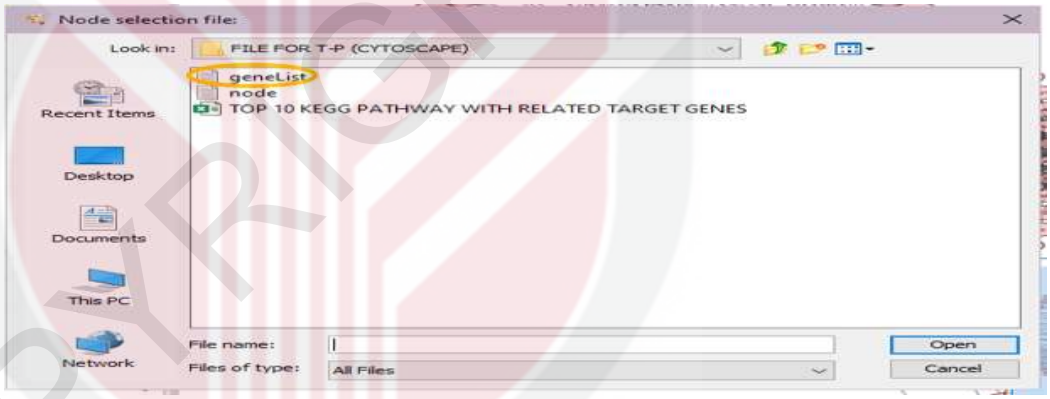
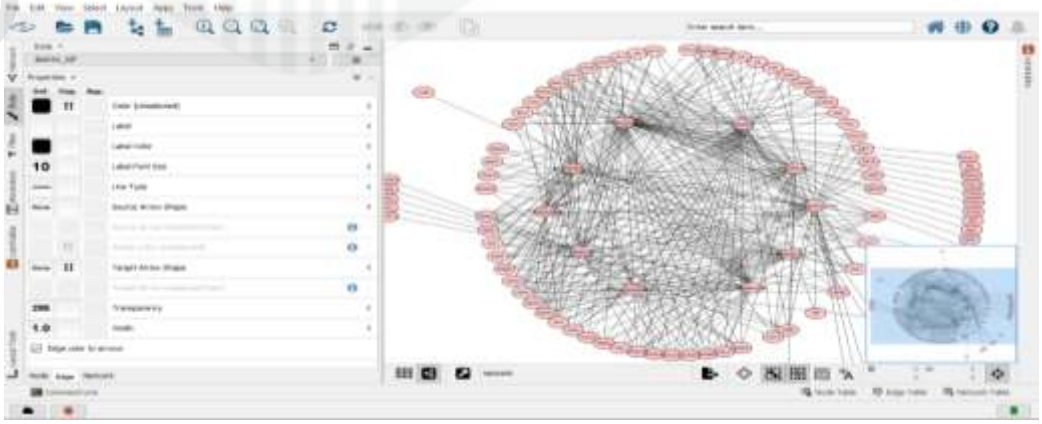
- We can also change the layout. Like the figure below is layout > circular layout



- To put the 10 pathways in the middle, go to select > nodes > from ID List File and choose geneList file and click open



- Then it will show like this after move the pathway to the middle



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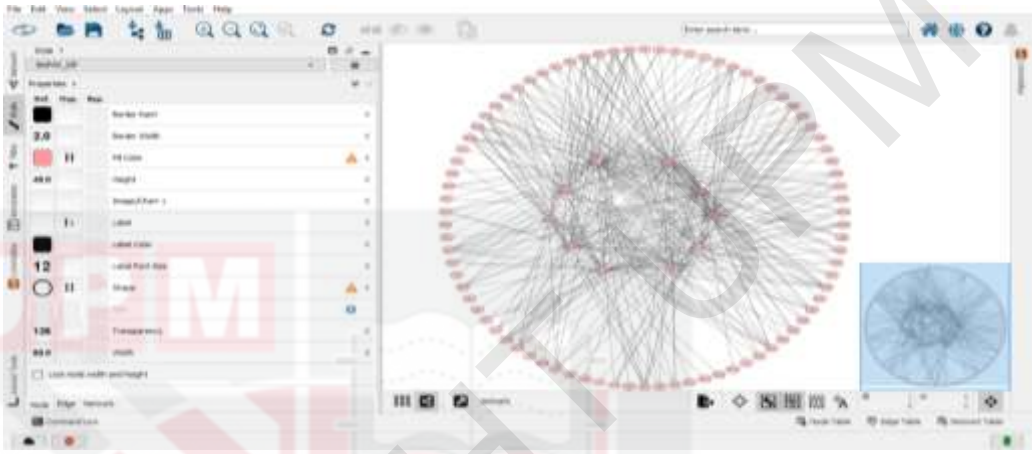
- To arrange to complete circle, go to select > nodes > from ID List File and choose geneList file and click open and then go to layout > attribute circle layout > selected nodes only > names and the result will give like this



- Before proceed with editing the nodes, we need to import the nodes.txt file to provide information for the Cytoscape by clicking file > import > table from file

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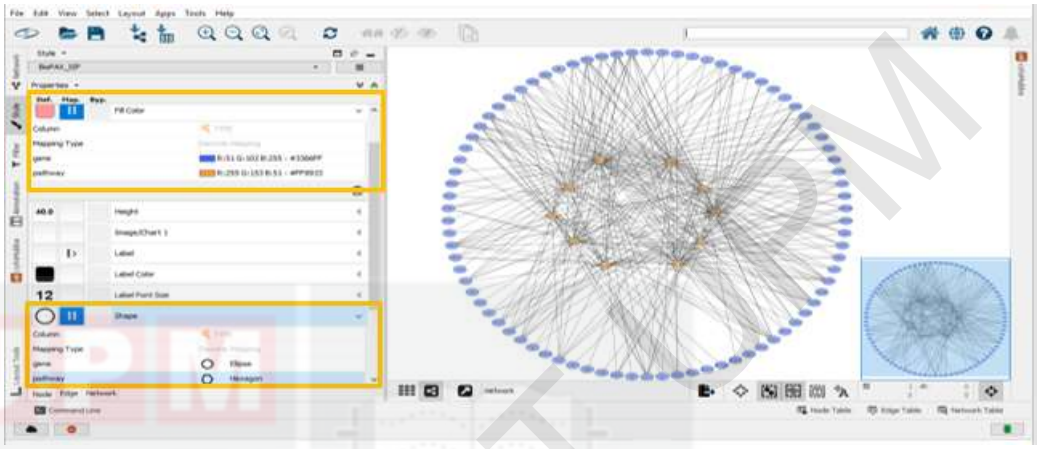
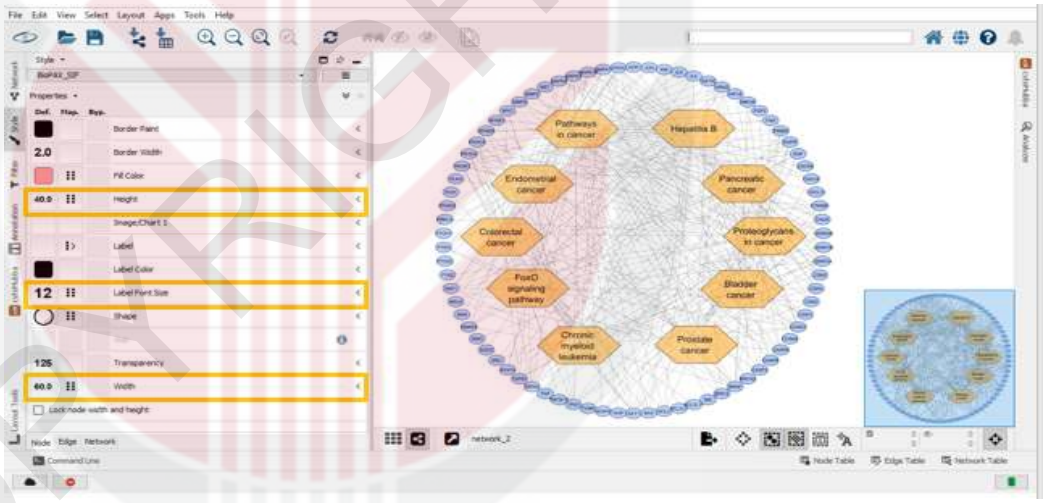
- Choose different fill color and shape for pathways and genes to differentiate between them by editing the fill color and shape like shown in the yellow boxes below here



- Can also change the height, font size and width of the nodes



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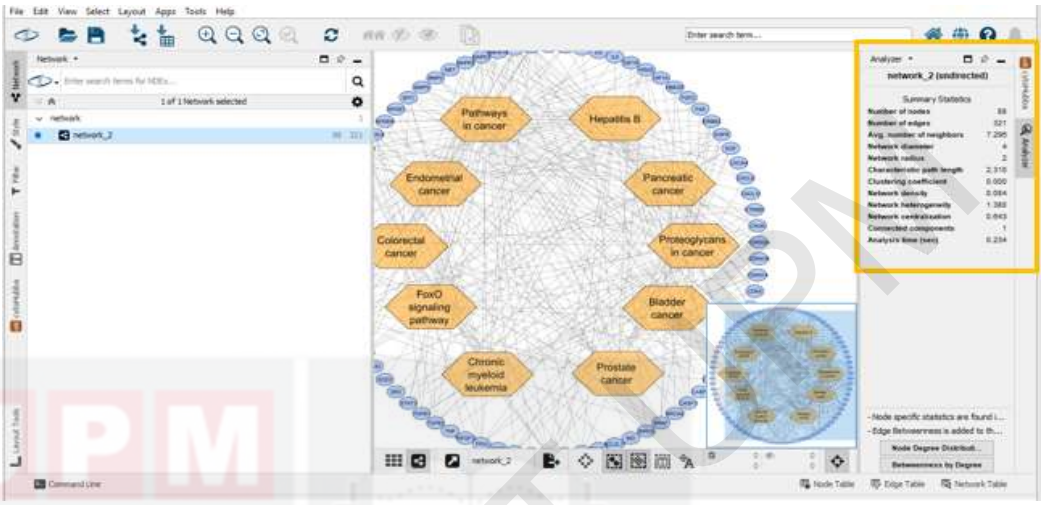
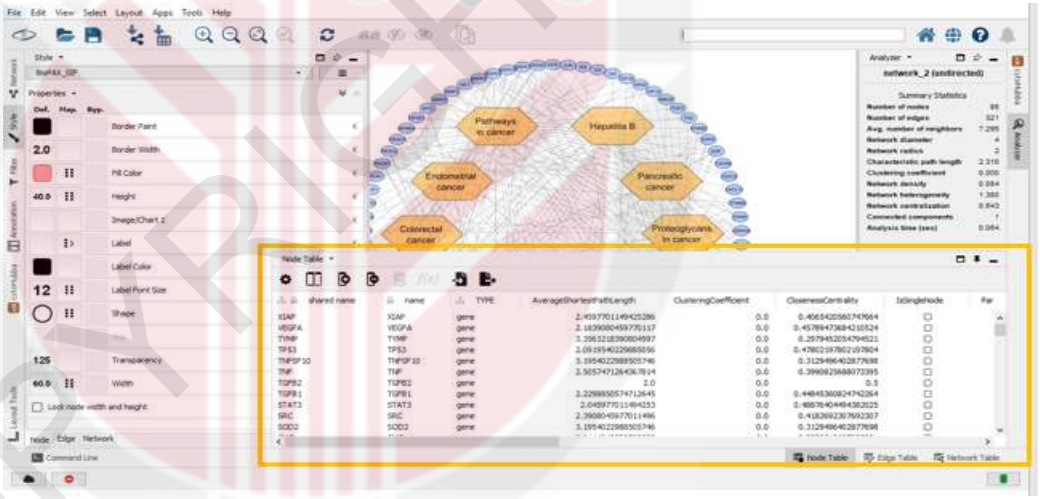
- To see the result of the analysis, click tool > analyze network. The result will be appeared on the right side



- The node information can also be downloaded in Excel format



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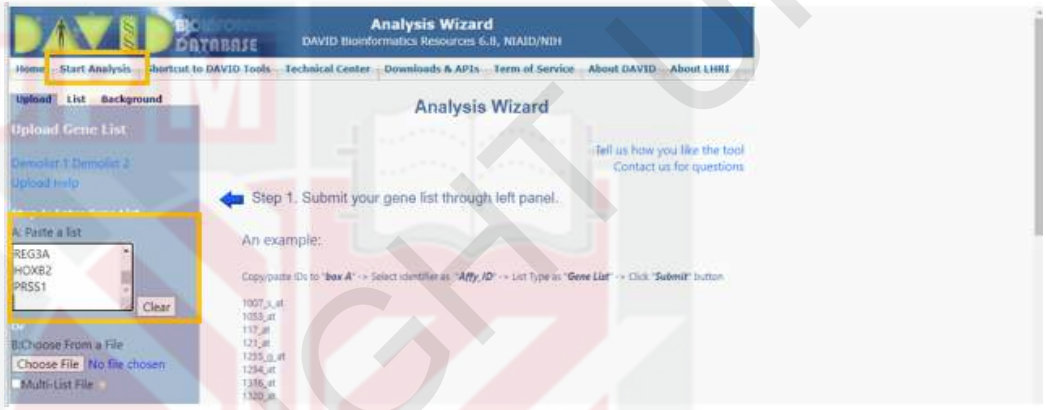
APPENDIX VIII

Gene Ontology and Pathway Enrichment Analysis

1. Gene Ontology Enrichment Analysis

- Go to DAVID Bioinformatics Resources 6.8: https://david.ncifcrf.gov/, choose start analysis and paste the overlapping genes



- Select identifier as Official_Gene_Symbol, species as Homo sapiens, list type as Gene List and click Submit button

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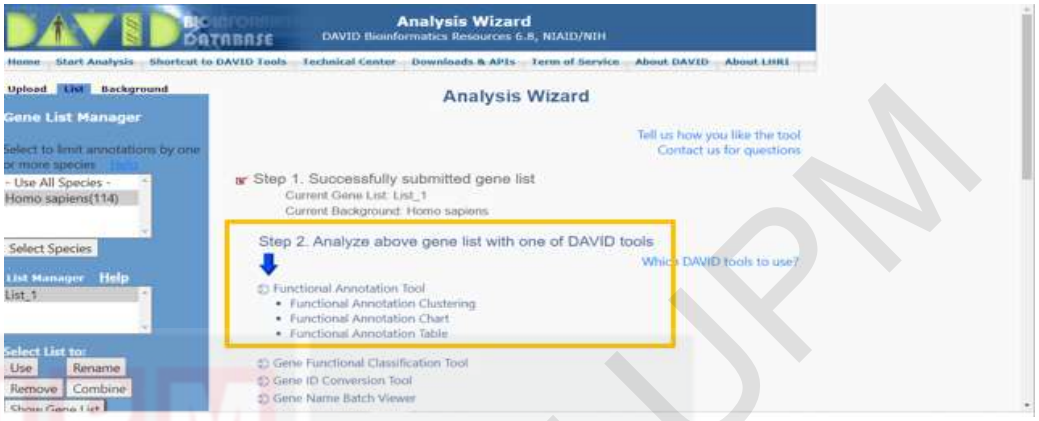
- . Start doing the analysis by clicking Functional Annotation Tool



- It will show like this

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

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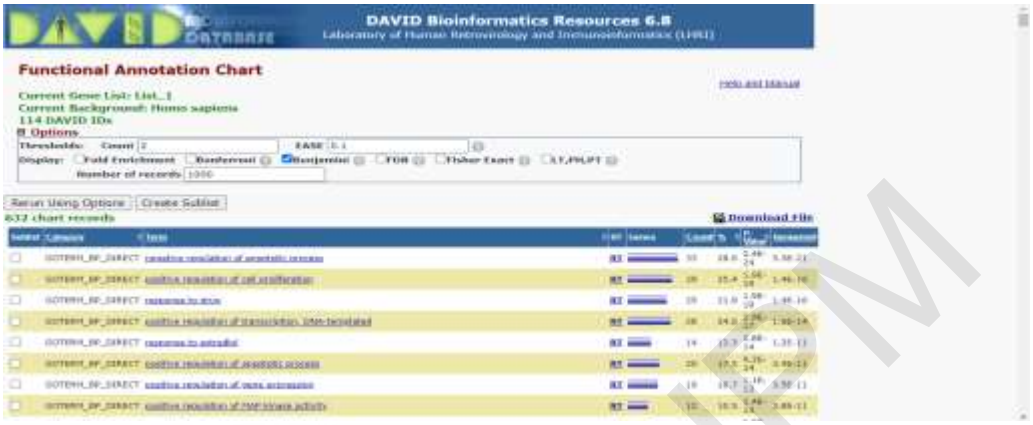
- The result will be appeared, and can be downloaded



2. KEGG Enrichment Analysis

- Similar step as performing gene ontology. But, from this page, we will choose pathway

- Go to KEGG Pathway and click chart to obtain the result

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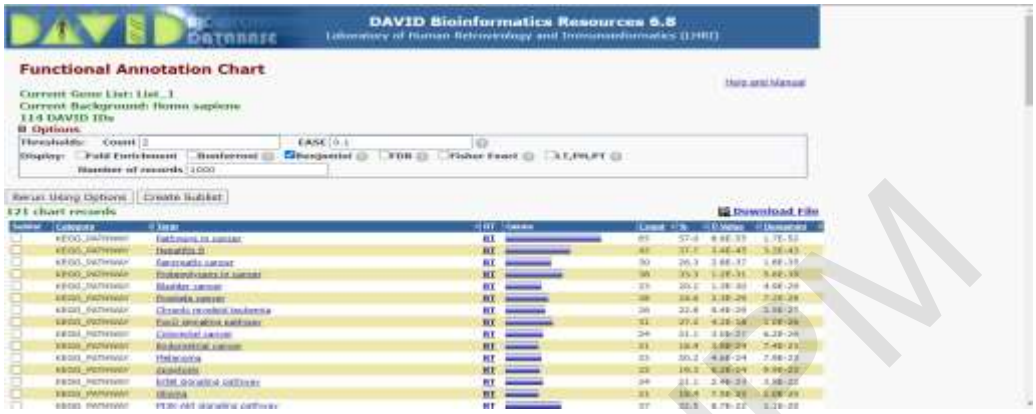
- The result will be appeared like this and can be downloaded



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