Identifying the Mode of Action of Lemon Grass (*Cymbopogon Citratus)* against Gastric Cancer Associated with *Helicobacter Pylori* Infection

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Abstract

***Purpose*:** Gastric cancer can be produced by environmental factors (radiation exposure, Smocking, *H. Pylori* infection) and also genetic factors. Lemongrass (*Cymbopogon citratus*) has long been a therapeutic and medicinal plant, used for its many beneficial properties. It has been used in various studies to treat stomach cancer.

***Methods*:** The identification of activity, therapeutic potential and screening of numerous target molecules of *Cymbopogon citratus* related to stomach cancer are studied with its methodical literature analysis. Shiny GO was used for Gene Ontology (GO) enrichment analysis and is designed with multiple functions. (1) gene signatures and enrichment results of graphical visualization, (2) a large annotation database from GO.

***Results*:** A literature review revealed that Cymbopogon citratus had 322 potential targets and 5 relative targets, 4 components, we also examined the relevance of these potential targets to gastric cancer therapy.

Conclusions The network pharmacology analysis revealed multiple biological pathways and target protein interactions which gave evidence through this research that *Cymbopogon citratus* had potential for the cure of gastric cancer.

Keywords: Biological effects; Shiny GO; Gastric cancer; Identifying the Mode of Action of *Cymbopogon citratus*; STITCH; TCMSP.

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1.Introduction

The third most common cancer type and the second leading cause of death worldwide is gastric cancer. Because gastric cancer was often discovered at an advanced stage, treatment was less

successful and resulted in median overall survival. A recent study was conducted to investigate epigenetic inhibitors of gastric cancer cell proliferation. The link between gastric cancer growth and development was shown by the critical role of epigenetic modifications in gastric cancer growth. (sri Renukadevi Balusamy *et al.*,2019) *Helicobacter pylori* (*H. pylori*) is a Gram-negative, microaerophilic bacteria in a spiral shape with four to six flagella that has an ecological niche in the human stomach. *Helicobacter pylori* (*H. pylori*) infects almost 50% of world's population. In 1994 WHO was classified *Helicobacter pylori* as a Group I carcinogen and cause gastric and duodenal ulcers. The pathogenesis of *Helicobacter pylori* was mainly due to pathogenic factors like urease, cytotoxin-binding gene antigens (Cag A), flagellum, vacuolating cytotoxin A (VacA), and others. (Yuan-Chuen Wang.,2014)

Conventional Chinese herb called dandelion containing complex active componds including polysaccharides, flavonoids, terpenes, pigments, phytosterols, coumarins, organic acids, etc. It plays an important role in many biological actions of organisms. Active compounds often inhibit the apoptotic protein expression, regulate cell cycle and cancer cell signaling, migration, and cancer cell proliferation, and effectively prevent the progression and tumor development. (Jumin Xie *et al.*,2020)

A recent study used one of the traditional Chinese herbs *Cymbopogon citratus* (DC) *Cymbopogon citratus* (DC) Stapf, commonly known as lemongrass, was used in Brazilian folk medicine to treat stomach cancer It was an aromatic plant. (Larissa Vezon et al., 2018) It had associated with reactive oxygen species (ROS)-related atherosclerosis, heart disease, cancer, and arthritis. It can be used to prevent several life-threatening diseases, such as: role decisive role. ROS generation had also been implicated in diseases of the gastrointestinal tract. (sri Renukadevi Balusamy *et al.*,2019)

Chemotherapy drugs can be used to treat gastric cancer, including (Capecitabine, Carboplatin, Cisplatin and Docetaxel) (Kyung Lee *et al.*, 2018). The mechanism of action of *Cymbopogon citratus* is unknown in gastric cancer, so we performed this research to identify the part of *Cymbopogon citratus* in cure for gastric cancer.

2. Methodology

The TCMSP is an advanced step in Traditional Chinese medicines System pharmacology that apprehensions the relationship between treatments, targets and illnesses (https://tcmspw.com). The proportion of unaltered medicines is termed as the oral bioavailability (OB). The development of PPIN, Gene Ontology (GO) and enhancement study was done by following this step.

Shiny GO is an instinctive, graphical web software that was used for Gene Ontology Enrichment Analysis, with the help of this website we create Gene Id, Enrichment Network Analysis, Tree, KEGG signaling Pathway and also develop a Plot for Gene Ontology Enrichment Analysis. (Steven Xijin Ge et al.,2020)

2.1. *Reclamation of Chemical Ingredients and Targets*

In this literature, with the help of TCMSP, all the chemical components of the herbs were checked first (Cheng *et al.*, 2017). This step is conducted by the analysis of compounds of *Cymbopogon*

citratus on the TCMSP database. After this stage, the herb related protein targets were recovered from TCMSP database (Ru *et al.*, 2014). These target proteins are then examined for functions related to performance or gastric cancer treatment (DING *et al.*, 2021).

2.2. Network Construction and Analysis

The selected genes were uploaded into the STICH database to get protein-protein interaction (PPI) information to explain the interaction between attacked genes. The desirable minimum interchange score was set to 0.400 condensation medium, remaining confines were left at their neglect values. The PPI data was then downloaded and presented with Shiny GO v0.741 (Chen *et al.*, 2020).

2.3. Pathway Enrichment Analysis and Gene Ontology

By structurally and hierarchically analyzing target genes using GO enrichment analysis, depending upon biological words, the features of the biological properties of the prospective attacked had been found or assessed (Cheng *et al.*, 2017). The gastric cancer potential of related proteins was investigated by a Gene Ontology Enrichment study. This is done using Shiny GO v 0.741. (Steven Xijin Ge et al., 2020)

3. Results

3.1. Reclamation of Chemical Ingredients and Their Targets

The enlistment of phytochemical compounds from literature search revealed some components in *Cymbopogon citratus,* which yielded the presence of 322 potential targets (Table 1) and 5 relative targets to the herb and gastric cancer in *Homo sapiens* (Table 3.2).

Table3.1. Active ingredients of *Cymbopogon citratus* with therapeutic effect against gastric cancer

Mol ID	Molecule Name	M.W	OB	DL
MOL000006	luteolin	286.25	36.16	0.25
MOL000765	Citraurin beta	432.70	20.53	0.56
MOL000498	isoorientin	448.41	23.30	0.76
MOL000773	cymbopogne	426.80	29.95	0.77

Table3. 2. Protein Targets of *Cymbopogon citratus* ingredients associated gastric cancer

Target ID	Target Name	Disease Name
1144	Hepatocyte growth factor receptor	Gastric Cancer
1939	Heat shock protein HSP 90	Gastrointestinal Stromal Tumors (GIST)
2203	Macrophage metalloelastase	Gastro-intestinal ulcers
290	Prostaglandin G/H synthase 2	Peutz-Jeghers syndrome
904	Glutathione S-transferase P	Gastrointestinal Neoplasms

3.2. Network Construction and Analysis

STITCH database was performed for the manufacture of complex protein network (Fig. 1), It consisted of the following association of three protein targets with an average network probability value of 0.400 with 51 edges and 13 nodes. End point of PPIN comprised of 10 functional interactions. The details of the created network demonstrate its p-value to be 1.49e-06. The important variety of edges in a network and the non-random choice signifies Small p-value. In this PPIN, we find that the order values and cluster coefficient i.e. 0.836, respectively. Here we see that the inflammatory process of EGFR is the largest. This is followed by other proteins such as UBC, HGS and GRB2. It shows the many functions contained in this protein, such as activation, inhibition, binding, catalysis, modification after translation, and its expression.

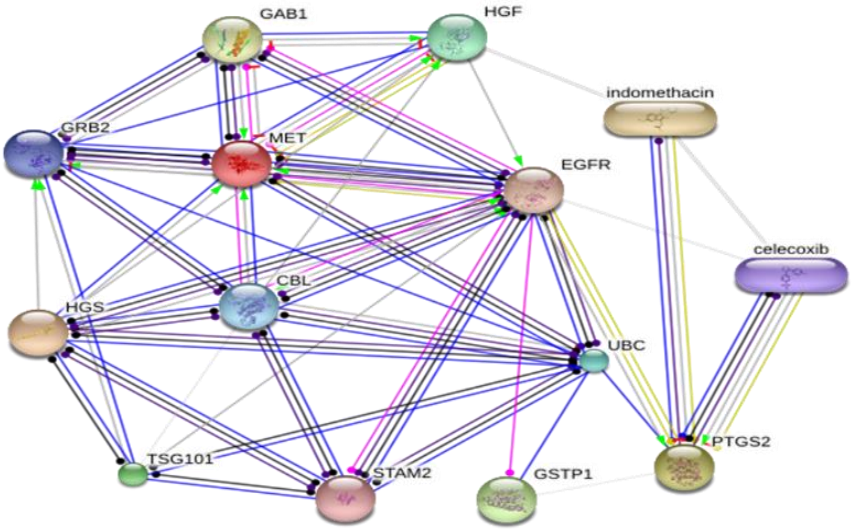


Figure: 1 Action view of the protein network of *Cymbopogon citratus* targets

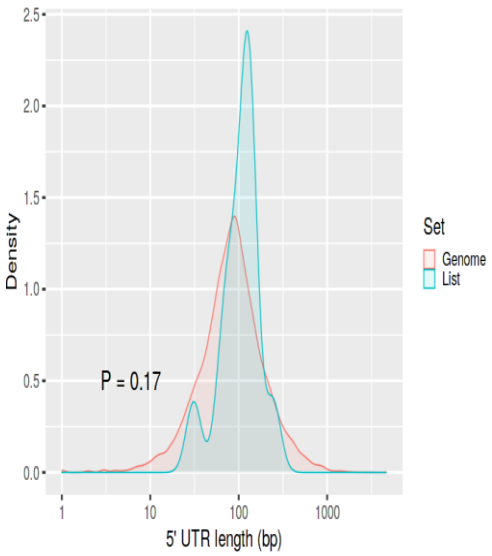
Table 3.3 GO terms and their related genes obtained through Shiny GO

Enrichment FDR	nGenes	Pathway Genes	Fold Enrichment	Go Term	Genes
4.80E-11	7	121	109.8	Epidermal growth factor receptor signalling pathway	TSG101 GAB1 CBL STAM2 EGFR GRB2 HGS
4.80E-11	6	47	242.5	Negative regulation of epidermal growth factor receptor signalling pathway	TSG101 CBL STAM2 EGFR GRB2 HGS
4.80E-11	6	52	219.1	Negative regulation of ERBB signalling pathway	TSG101 CBL STAM2 EGFR GRB2 HGS

9.57E-11	7	143	92.9	ERBB signalling pathway	TSG101 GAB1 CBL STAM2 EGFR GRB2 HGS
1.68E-10	11	1411	14.8	Negative regulation of signal transduction	HGF PTGS2 TSG101 GSTP1 MET CBL STAM2 EGFR UBC GRB2 HGS
3.13E-10	11	1537	13.5	Negative regulation of cell communication	HGF PTGS2 TSG101 GSTP1 MET CBL STAM2 EGFR UBC GRB2 HGS
3.13E-10	11	1540	13.5	Negative regulation of signalling	HGF PTGS2 TSG101 GSTP1 MET CBL STAM2 EGFR UBC GRB2 HGS
4.43E-10	6	87	131.0	Regulation of epidermal growth factor receptor signalling pathway	TSG101 CBL STAM2 EGFR GRB2 HGS
6.34E-10	6	94	121.2	Regulation of ERBB signalling pathway	TSG101 CBL STAM2 EGFR GRB2 HGS
7.29E-10	10	1122	16.9	Enzyme linked receptor protein signalling pathway	HGF TSG101 MET GAB1 CBL STAM2 EGFR UBC GRB2 HGS
1.37E-09	9	762	22.4	Transmembrane receptor protein tyrosine kinase signalling pathway	HGF TSG101 MET GAB1 CBL STAM2 EGFR GRB2 HGS
2.30E-09	11	1940	10.7	Negative regulation of response to stimulus	HGF PTGS2 TSG101 GSTP1 MET CBL STAM2 EGFR UBC GRB2 HGS
5.03E-09	7	296	44.9	Biological process involved in interaction with host	TSG101 MET CBL EGFR UBC GRB2 HGS

1.05E-08	12	3363	6.7	Cell surface receptor signalling pathway	HGF PTGS2 TSG101 GSTP1 MET GAB1 CBL STAM2 EGFR UBC GRB2 HGS
1.05E-08	12	3361	6.7	Regulation of signal transduction	HGF PTGS2 TSG101 GSTP1 MET GAB1 CBL STAM2 EGFR UBC GRB2 HGS
2.87E-08	7	391	34.0	Biological process involved in symbiotic interaction	TSG101 MET CBL EGFR UBC GRB2 HGS
3.53E-08	12	3759	6.0	Regulation of cell communication	HGF PTGS2 TSG101 GSTP1 MET GAB1 CBL STAM2 EGFR UBC GRB2 HGS
3.56E-08	12	3797	6.0	Regulation of signalling	HGF PTGS2 TSG101 GSTP1 MET GAB1 CBL STAM2 EGFR UBC GRB2 HGS
3.56E-08	8	722	21.0	Cellular response to growth factor stimulus	HGF GSTP1 GAB1 CBL EGFR UBC GRB2 HGS
4.39E-08	8	750	20.2	Response to growth factor	HGF GSTP1 GAB1 CBL EGFR UBC GRB2 HGS
4.26E-07	12	4707	4.8	Regulation of response to stimulus	HGF PTGS2 TSG101 GSTP1 MET GAB1 CBL STAM2 EGFR UBC GRB2 HGS
4.33E-07	10	2318	8.1	Vesicle-mediated transport	HGF TSG101 GSTP1 MET CBL STAM2 EGFR UBC GRB2 HGS

1.79E-06	5	188	50.5	Positive regulation of protein kinase B signalling	HGF MET GAB1 EGFR GRB2
3.17E-06	3	15	379.9	Entry of bacterium into host cell	MET CBL GRB2
3.20E-06	7	824	16.1	Regulation of kinase activity	HGF TSG101 GSTP1 MET CBL EGFR HGS
3.50E-06	8	1355	11.2	Regulation of phosphorylation	HGF PTGS2 TSG101 GSTP1 MET CBL EGFR HGS
3.50E-06	5	222	42.7	Movement in host environment	TSG101 MET CBL EGFR GRB2
3.59E-06	9	2057	8.3	Biological process involved in interspecies interaction between organisms	PTGS2 TSG101 GSTP1 MET CBL EGFR UBC GRB2 HGS
6.01E-06	5	251	37.8	Regulation of protein kinase B signalling	HGF MET GAB1 EGFR GRB2
6.97E-06	7	948	14.0	Regulation of transferase activity	HGF TSG101 GSTP1 MET CBL EGFR HGS



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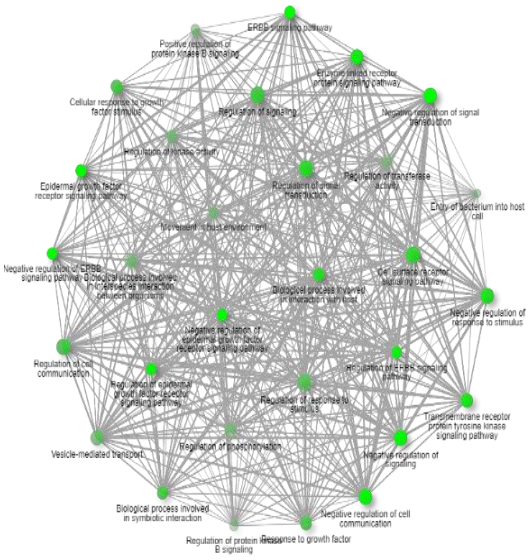

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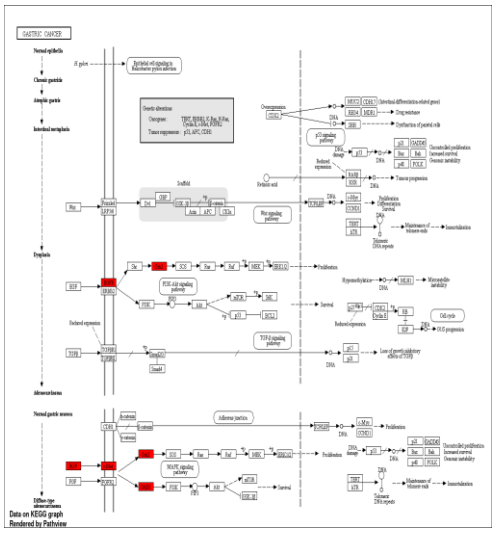

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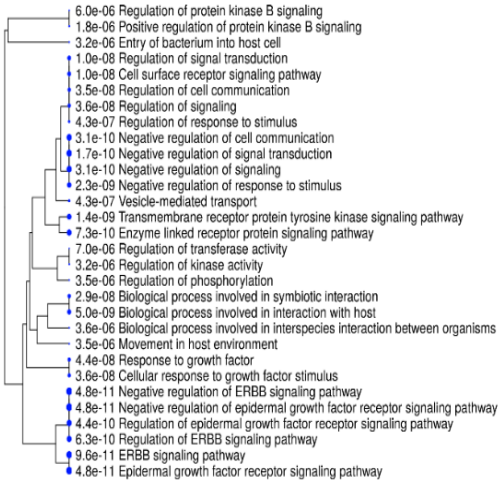

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Figure 2. Example outputs of Shiny GO. (A). Distribution of the lengths of 5' UTRs in query genes vs. other coding genes in the gene. (B). Enriched GO molecular component terms visualized as a network. (C). A partial KEGG pathway diagrams with genes highlighted. (D). Hierarchical clustering tree.

4.Discussion

A pharmacological network approach had been developed and adapted to elucidate the potential for gastric cancer in *Cymbopogon citratus*. The study included a variety of methods including PPIN construction, gene enrichment analysis, and a total of 322 potential protein targets were observed. In addition, analysis of pathway enrichment revealed signs of regulation of *Cymbopogon citratus*, a number of signaling pathways that promote gastric cancer and cancer of Homo sapiens. In this study, the presence of several components such as luteolin, isoorientin, Citraurin beta, cymbopogne, in exhibiting the bioactivity of *Cymbopogon citratus* gastric cancer.

Luteolin is a widespread dietary flavonoid with various useful biological functions like luteolin, stimulate apoptosis in gastric cancer and significantly preventing proliferation, cell cycle progression, migration, colony formation, invasion. (Yansong Pu et al.,2018) The β-catenin signaling pathway is involved in a significant proportion of gastric cancer development and progression. (Miguel Angel Chiurillo et al., 2015) Ntural flavonoid is a Isoorientin (ISO), had 6-C-glucoside of luteolin. In addition, ISO suppressed the pathogenic genera of *Helicobacter* that can cause inflammation and the growth of most bacteria in the gut microbiota. (Li Yuan et al., 2018) Expression of human macrophage metalloelastase (HME) mRNA and protein plays role in evaluation of their role in gastric cancer development, gastric cancer cell lines and correlation with patient prognosis. (H Zhang et al., 2007) Cyclooxygenase also known as Prostaglandin endoperoxide synthase, expression was upregulated in gastric cancer and its molecular mechanisms

are being studied. *Helicobacter pylori* infection, nuclear factor kappa B activation and tumor suppressor gene mutations can be responsible for the over expression of COX-2 in gastric cancer. (Jiancheng et al., 2013) Molecular chaperone heat shock protein 90 (Hsp90) has attracted attention as a promising target for anticancer drugs because it is important for maintaining the stability, integrity, conformation, and function of major carcinogenic proteins. Hsp90 is often upregulated in gastric cancer (MOSER *et al.*, 2009). Glutathione S transferase placental morphology (GST) in cancerous tissues of the colon and esophagus was determined by a single radial immunodiffusion or activity inhibition test (K Sato*et al.*, 1989). Cell proliferation and infiltration is caused by Hepatocyte growth factor (HGF) which in turn causes malignant cancer e.g.; Gastric cancer. (Ae Koh., 2020)
Genetics and enrichment analysis showed the relativity between all GO terms and stomach cancer. In this research done to evaluate deciphering the role of *C. citratus an* against gastric cancer pharmacologically. This makes them dependent on various phytochemicals that are active in this mechanism.

Conclusions

In network pharmacology, various aspects of the mechanism of action of a specific plant by studying various characteristics of plant pharmacology and pharmacodynamics. Modern databases and systems biology analyses are used to clarify the mechanism of action of various herbs Such as C. *citratus* essential oil has potential in treating stomach ulcers and preventing stomach cancer. *C. citratus* is essential to prove its therapeutic activity in gastric cancer associated with *H. pylori* infection and rehabilitation.

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