" *IDENTIFICATION OF PHYTOCHEMICALS OF GINGER AS EFFECTIVE IMMUNOMODULATOR USING GENE ENRICHMENT ANALYSIS*"

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE
OF

MASTER OF TECHNOLOGY

IN

BIOINFORMATICS

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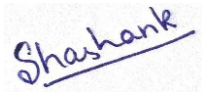
CANDIDATE'S DECLARATION

I, Shashank Kumar Singh (Roll No.: 2K19/BIO/04), student of Master Of Technology in Bioinformatics, hereby declare that the project dissertation titled "**IDENTIFICATION OF PHYTOCHEMICALS OF GINGER AS EFFECTIVE IMMUNOMODULATOR USING GENE ENRICHMENT ANALYSIS**" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship, or other similar title or recognition.



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This is to certify that the M.Tech. Synopsis entitled "**Identification of phytochemicals of ginger as effective immunomodulator using gene enrichment analysis**" submitted by **SHASHANK KUMAR SINGH (2K19/BIO/04)** in partial fulfillment of the requirement for the award of the degree of Master of Technology from Delhi Technological University, is an authentic record of the candidate's own work carried out by him under my guidance.

Place: New Delhi
Date: 29 July,2021

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CERTIFICATE

This is to certify that the M.Tech dissertation entitled "**Identification of phytochemicals of ginger as effective immunomodulator using gene enrichment analysis**" in partial fulfillment of the requirement for the award of the degree of Master of Technology in Bionformatics submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my own work, carried during a period from 7-Jan-2021 to 28-July-2021. The information and data enclosed in this dissertation is original and has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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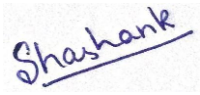

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ABSTRACT

Cancer is one of the most prevailing diseases across the globe. Despite the advancements in the multitude of therapies, we are unable to find an efficient, cheap and more precise treatment mechanism for multiple cancers. In order to handle cancer, an integrated strategy is required. Scientific knowledge has been developing, and this understanding is critical to the success of treating cancer. Many herbal and traditional components are now being researched to verify their ability to inhibit the growth of cancer cells. Herbal medicines have been traditionally used for years for the cure of numerous diseases. Ayurveda is said to provide a new step on to the medical benefits of cancer in the form of characteristics seen in arbuda and granthi, two diseases described in the Sushrutha Samhita. So in this study, the treatment for cancer outlined in Ayurveda is discussed in an effort to identify the herbs used for therapy and having activity proved by experimentations. Ayurvedic pharmaceuticals found in the literature on cancer medications and those that are new to anticancer drug development were identified. All of these studies build upon centuries of ancient Ayurvedic research. Ginger is amongst the most utilised medicinal plants in food, beverages and medicine. Additionally, findings from trials using single and compound Ayurveda remedies that are intended to elucidate their anticancer activity further reinforce the notion that Ayurveda therapies are powered by science and not just a random approach.

Although earlier studies have extensively shown anti-cancer and anti-inflammatory activity, many of its phytochemicals have not been explored extensively which may be useful in combinatorial therapies. In the present study, using bioinformatic tools we have performed gene enrichment analysis using a gene set having all known genes with whom ginger phytochemicals interact and its validation using the experimental dataset from NCBI GEO. Thus, providing us with a better idea of the immuno-modulatory role of ginger along with its other previously known activities. Ginger phytochemicals were found to be interacting with a significant number of genes that are engaged in regulating pathways of immune responses like inflammatory responses, adaptive immune responses and apoptosis. Using this immunomodulatory property of ginger, it can be used in cancer treatment therapies, thus proving to be cheap and less damaging cancer therapy.

Keywords - Cancer therapy; Immunomodulator; Ginger; Gene Enrichment Analysis; Natural compounds

CHAPTER-1 INTRODUCTION

Cancer is defined by uncontrolled multiplication of cells in the human body resulting in a further complication of secondary outgrowths of malignant cells with the ability to metastasize [1]. Among all different types of cancers, breast cancer remains one of the major contributors of deaths among women worldwide [2]. Cancer treatments consist of radiotherapy, chemically generated drugs and chemotherapy. Chemotherapy is exorbitant for patients and comes with many negative side effects. Current difficulties relating to efficient cancer management are drug resistance, continuous increase in tumour heterogeneity and bystander tissue damage [3]. Irrespective of great advancements made towards the advancement of conventional chemotherapy and the understanding of disease pathophysiology, effective cancer management is still a work in progress.[4]. Thus, there is a lot of focus on the use of alternative cancer therapies and treatments [5]. This necessitates a growing need to develop unique, reliable, low in cost, and target-oriented therapy approaches along with identifying new drug candidates.

For several years in developing countries, herbal remedies have always been the main source of medical therapy. Plant extracts were utilised in medicine for centuries because of their natural antiseptic characteristics. Traditional medicinal plants since ancient times have had a substantial impact on the treatment of a variety of cancers [6]. To cure or check the development of cancer many plant species are already being used. With extensive research work over the plants that are in use as herbal medicine in developing countries, plant species exhibiting anti-cancer properties are identified [7,8,9]. Necessary housekeeping genes of plants are being studied for their capacity to restrict development by initiating apoptosis in cancerous cells. Further, terrestrial plant extracts are being explored for possible nanomaterial based therapeutics for diseases like cancer [10]. Drug discovery and medicinal plants have considerably maintained a promise for treating cancer along with many human degenerative diseases. For the treatment of a range of cancers, several important bioactive compounds from plants like Taxol, Camptothecin, Vincristine, Vinblastine, Vinorelbine, Vindesine, Vinflunine etc. have apparently been established as of strong therapeutic importance [11]. Plant species like Taxus brevifolia, Taxus baccata, Catharanthus roseus and Podophyllum peltatum have been vastly identified for their ability to treat a variety of cancers[12].

Ginger has been used as an edible and medicinal plant for ages in India , but it is currently mostly employed in the cosmetics industry, as a spice, and as a raw ingredient for food.. In recent decades, its many bioactive components involving gingerols, polyphenols, polysaccharides, and essential oils, have attracted interest [13]. According to studies, ginger

shows a vast range of biological properties, like anti-inflammatory [14], antioxidant [15], anti-cancer properties [16], and so on.

In the last two decades, Ginger polysaccharides have attracted much attention which is among the ginger's most crucial bioactive substances. Bioactivities such as antioxidant [17], anti-tumour activities [18], anti-coagulant [19], and primary structure characterization including molecular weight and monosaccharide composition[20] have been reported. Furthermore, the main strategy is to alter the immune system's ability to attack cancer cells indirectly [21].To our knowledge, there has been very little study on the immunomodulatory action of ginger polysaccharides.

In the present study, we curated all phytochemicals of ginger, explored their interactions with genes in the human genome and finally using the resulting gene set we performed gene enrichment analysis. We also verified the enrichment result from datasets of cancer cell lines exposed to ginger from the NCBI GEO database. This will help us have a cumulative idea for the overall effects of ginger in devising novel treatment of cancer.

CHAPTER-2 REVIEW OF LITERATURE

In the 21st century, cancer is one of the most feared illnesses. Currently, it is the leading source of mortality in the world, which responsible for 25% of all deaths. Development and an evolved style of cultural and social life driven by Modern medicine are seen as adversaries of each other. The interdisciplinary scientific community is putting every attempt to combat this sickness, but an absolutely positive, flawless remedy has not yet been provided to the global health community. More currently, research attention has been placed on studies dealing with adjunctive therapies for the treatment of cancer. Many research investigations have been done on the various ethnobotanical basis of plants. For instance, Hartwell [22]-[30] has tracked down and studied roughly 3,000 different plants with anticancer qualities, and these plants have since been employed as effective anti-cancer medications. Ayurveda, an ancient Indian system of plant-based therapy, has successfully used these natural remedies and methods to deal with different cancers for centuries. The main objective of this article is to offer a comprehensive overview of many types of cancers and their treatment, as well as the many scientific ideas that go into their treatment, from an ayurvedic viewpoint using natural materials. This article discusses studies on ayurvedic anti-cancer herbs and also provides an overview of cancer therapy approaches. The aim of this document is to heighten knowledge of and get people excited about ayurvedic cancer remedies, as well as advocating an integrated strategy to fighting cancer that incorporates both standard and ayurvedic treatments.

2.1. Fundamental classification

The Ayurvedic system of classifying neoplasms takes into account a range of clinical signs and characteristics in connection to the Tridoshas.

- **Group I:** Ailments which could be termed as clear malignancy, which includes arbuda and granthi, e.g. mamsarbuda (melanoma) and raktarbuda (leukaemia), mukharbuda (oral cancer), etc.
- **Group II:** Ailments which could be termed as cancer, such as incurable ulcers with e.g. tridosaj gulmas (abdominal tumours like carcinomas of the stomach and liver or lymphomas).
- **Group III:** Ailments which have probability of malignancy, e.g. Visarpa (erysipelas), asadhya kamala (incurable jaundice) and nadi vrana (sinusitis) [31,32].

2.2. Cancer therapy—a practical dilemma

Resolving this difficult issue is crucial in helping to stop the spread of this terrible illness. A way of dealing with the issue of western medicine using medicinal plant extracts, that has many negative effects, is an alternative option that harnesses the virus-destroying ability of plant compounds. The function of numerous herbs that have been researched in clinical research is being further studied via the use of laboratory techniques to determine their tumour-specific cytotoxic effects. As a result, patients who are already suffering with cancer have had their condition exacerbated by medication-induced toxic side effects, which has caused them to turn to complementary and alternative medicine in the hopes of finding a cure. According to Ayurvedic medical theory, a superior method of curing these chronic ailments has previously been unable to be successfully treated using western medical procedures. Many traditional Indian medicines with their lengthy development history have always caught the attention of practitioners and researchers for their uses in cancer therapy on a scientifically sound study basis.

2.2.1. Principles of ayurvedic treatment

Illegal or irresponsible usage of nature's law may disturb the human system and result in sickness like cancer. This time, it is the most prominent physician, who is once again, bringing the cure. The Ayurvedic system of medicine was established on fundamental principles of nature and the constituents. After a comprehensive examination of human physiology, the concepts were validated. This is the first approach to highlight health as the condition of being ideal in all five areas of physical, psychological, social, and spiritual well-being for humans. Namaste Healing: it is broken down into four types as basic Prakritistapani therapy (health maintenance), Rasayan treatment (cure of sickness), Rasayana treatment (restoration of normal function), and Naishtikchikitsa (spiritual approach) [33]. An Ayurvedic practitioner aims to identify the origin of sickness in order to aid the recovery process. The process of illness development is divided into six phases, including the early stages, in which an exacerbation, an accumulation, an overflow, a shift, a buildup in a new area, and a manifestation of a recognised illness occurs. An Ayurvedic physician can identify whether there is a problem at the early stages of an imbalance in the body and their treatment method ensures an equilibrium

by delivering required components as well as limiting the excessive ones. Surgery is only considered in the most severe situations.

Herbal decoctions often used in Ayurveda that include many plants each with considerable promise for cancer treatment are known as ayurvedic herbal decoctions. These formulations are said to affect various biochemical pathways and are capable of having a substantial impact on various organ systems. The benefits of a herbal decoction include being able to feed the whole body as it supports a variety of organ systems [34]. This list includes several herbs that have been scientifically confirmed to have anti-tumour effects and are used to treat a variety of malignancies.

- Derived and separated diterpenes (andrographiside and neoandrographolide) have been shown to be effective against tumorigenesis because of their ability to inhibit lipid peroxidation and to promote carcinogen detoxification [35-38].
- Using acetogenin found in Annona atemoya, a small compound that was first discovered in 2013, it has been shown that it causes the body to undergo apoptosis and seems to lead to the chromatin margination and condensation of cancer cells. In addition to annonaceous acetogenins, e.g. muricins A-G, muricatetrocin A and B, longifolicin, corossolin, and corossolone, several other annonaceous acetogenins, e.g. anoicins A-G, anonacicatrocin A and B, longifolicin, and corossolone, were found to be highly selective in increasing in vitro cytotoxicity to tumour cells [39].
- It was discovered that aqueous extracts of P. amarus were able to lengthen the life span of the tumour-bearing rats, as well as to normalise glutamyl transpeptidase activity [40]. Theory suggests that HBsAg mRNA transcription and post-transcription is disrupted in viral carcinogenesis, and this could be beneficial for the development of new treatments for HBV infection [41].
- Piperine, an active alkaloid derived from this plant has been employed as an element of ayurvedic anticancer medicines because of its ability to help combat oxidative stress in both in vitro and in vivo circumstances [42].
- Podophyllin and its active ingredient, podophyllotoxin, are recognised for their cytotoxic impact by virtue of their qualities of mitotic inhibition, nuclear fragmentation, defective spindle formation, they're also shown to be karyoplasm. It's a potent cancer-fighting medication against several tumours, for example, sarcomas, adenocarcinoma, and melanoma. Necrosis is believed to be the primary mechanism of action. It is thought

to be caused by the cytotoxic impact of the drug on tumour tissues. Patent application procedures for these derivatives have been validated in cancer chemotherapeutic research, and these derivatives are exclusively produced using those techniques [43]. Since recently, the broad usage of chemically modified podophyllotoxins in cancer therapies has been occurring. Podophyllotoxin is the research name for etoposide, a podophyllotoxin derivative which has been investigated in laboratory and animal models, as well as against liver cancer in humans for more than a decade. Epirubicin was studied in conjunction with the solution, and so the solution has shown its effectiveness in Phase II trials [44,45]. up to 3% of the patients had a full cure, while up to 36% had a partial response to the treatment by means of this combination therapy. An analysis shows that P-glycoprotein, a drug efflux pump, does not have as significant an impact on lowering VP-16 concentrations in cancer cell lines, and hence this medication is more effective in these cells. As long as it is within the recommended dosage, the medicine is safe even when administered at higher doses [46].

- The active components from T. cordifolia work with the host immune system to increase the amount of immunoglobulin and blood leukocyte counts, and by stimulating stem cell growth. In the treatment of solid tumours, this drug is known to have a remarkable anti-tumour effect. Compared to it, chemotherapeutic agents like cyclophosphamide have the capacity to decrease tumour volume by 58.8 per cent [47]-[49]. In this case, the immunostimulating qualities might be employed to avoid tumour-mediated immunosuppression, which might lower the immune system. This may make a medication option for many malignancies.

2.3. Cancer therapy in Ayurveda — Its validation and modernisation

A huge population uses ayurvedic medicine due to its having tremendous efficacy around the world, leading to an even greater need for carefully conducted, high-quality intensive research on its efficacy and to further develop this discipline to meet the ever-new challenges of modern medicine in the field of oncology. the most severe review should be done using gold standards for clinical research, in the form of the randomised controlled clinical trial (RCT). When looking at it that way, research funding should be focused on clinical investigations in Ayurveda where studies are carefully planned, which display positive outcomes for conditions like cancer to whom western therapy has demonstrated to be less beneficial. Given the increase

in preventable illness and injury in recent years, as well as new advances in treatment methods, it is critical that providers not only evaluate the safety and efficacy of treatment strategies, but also consider aspects of community practise settings, patient expectations, compliance, and cost-effectiveness. In the development of low-cost herbal remedies, standardisation and the promotion of high-quality manufacturing may enable us to provide a product with less risk and greater efficacy than pharmaceutical alternatives. Although there will be plenty of interested parties for investigations on anticancer ayurvedic medications, investigations on such treatments will become more popular from an economic perspective, given that cancer is the most prominent cause of mortality today.

2.4. Future Perspective

The rising numbers of patients in recent decades who have sought out Ayurved as an adjuvant therapy option for different illnesses show how rapidly the treatment has gained traction. Ayurvedic Herbal Medicine, which has shown promising results for use as an adjuvant treatment after surgery, chemotherapy, irradiation, or other sorts of therapies for cancer patients across the globe, is becoming even more widely used. Herbal medicine is seen as a gift of nature, and chemicals found in herbs have the advantages of being readily available, having proven effectiveness, and is generally safe and non-toxic when compared to chemotherapy. In addition to Ayurved treatment, it is also established that the use of chemotherapy or radiation in conjunction with it is capable of improving effectiveness and reducing restrictions and downsides created by the use of chemotherapy or radiation. As a result, there has been a significant study conducted to clarify the processes behind the therapeutic benefits of Ayurved in cancer therapy. Cancer therapies have not eliminated all tumour cells. New theories suggest that these treatments could even be able to control oncogenes and tumour suppressor genes, epigenetic alteration, the microenvironment, and cancer stem cells. The aim of this study was to provide improved knowledge of Ayurvedic adjuvant treatment for cancer. It is no exaggeration to say that the number of research pertaining to curcumin and resveratrol in cancer treatment is bigger than all the other phytochemical components from Herbal medicine. That means there is a great deal to learn about these compounds. By studying more of the Ayurvedic medicinal ingredients, it will be possible to fully use Ayurvedic treatments in cancer treatment and get a wider acceptance of the use of Ayurvedic medicines.

In the current climate, a great deal of clinical effectiveness and toxicity of several anticancer medicines remain unclear and unclear. A good illustration of this is research into most of the

ayurvedic medications that are still in the pre-clinical stage or are not being pursued. Future studies that examine this area will assist in identifying safe and effective anticancer medications, and they will further the understanding of their mechanism of action. There is a great opportunity for Ayurvedic practitioners and researchers in medical sciences to assist develop this therapy by expanding their engagement and contribution. People no longer have the luxury of choosing to reject ayurvedic treatments or regard them as something unorthodox from standard medical techniques. The goal of modern treatment is to be certain and to use both logic and intelligence to do so.

CHAPTER-3 METHODOLOGY

3.1. Curation of the list of phytochemicals present in Ginger from IMPPAT.

IMPPAT is an Indian database for medicinal plants storing their phytochemicals along with their medicinal uses. It has data of around 1743 plants, 9597 phytochemicals, 1125 remedial uses along with 11515 and 27075 associations of plants with various phytochemicals and remedial uses. The redundancy of data is removed by manual curation along with their structural details and unique chemical identifiers. They use cheminformatic approaches to compute various properties of phytochemicals like drug-likeliness, distribution, excretion, absorption, toxicity, metabolism along with their physicochemical properties [50]. On the query search, for Zingiber officinale (Ginger) over IMPPAT we got a table of 64 phytochemicals(**Table 1**) present in Ginger.

3.2. Finding interactions of phytochemicals with genes in the human genome using CTD.

Started in 2004, the Comparative Toxicogenomics Database (CTD) is an open-access web-based database that enables the user to curate interactions of human health with various chemicals which in turn aid in the knowledge of illnesses caused by environmental factors. Its data is extracted by hand directly from scientific publications and further using standard terminology information regarding the relationship between compounds and various genetic aspects. Every interaction is footnoted with taxonomic designation (allowing for cross-species statistical correlation) and attributed to initial publications(providing reproducibility and clarity). All these efforts in turn improve the consistency and reliability of data and provide information normalization, consolidation, and accessibility. CTD offers its users easy to use interface, statistical models and unrestricted access to storable content [51]. CTD is available at http://ctdbase.org. We searched for interaction of 64 phytochemicals curated earlier with genes over human genome using CTD one by one and extracted protein-gene interactions for 20 phytochemicals and finally manually extracted a gene set of 72 genes along with their Gene IDs(Table 2) from downloaded result files.

3.3. Performing enrichment of gene list curated using Enrichr.

Enrichr is an efficient open access, web based tool for the study of data sets from genomic experiments. Enrichr holds a huge depository of diversified libraries of gene sets accessible for analysis and even download. Enrichr holds hundesd of annotated gene sets from more than 102 gene set libraries. It has been recently upgraded its latest features including option to upload BED files, uploading fuzzy data sets, clustergrams for improved representation of results and improved application programming interface [52]. Overall, Enrichr is a biological data based search engine that holds upto date developments details and an extensive source for gene set analysis. Enrichr is freely available at: http://amp.pharm.mssm.edu/Enrichr. Using Enrichr, we performed gene enrichment analysis using the earlier curated dataset of 72 genes and find out that majority of those genes were involved in pathways of cancer(32 genes) (Table 3) and apoptosis(22 genes), using KEGG 2019 HUMAN database. Result (https://maayanlab.cloud/Enrichr/enrich?dataset=aed1753c60ceedb899a084b3c3039ba5) (Figure 1).

3.4. Curation of an experimental dataset for validation from NCBS GEO.

Gene Expression Omnibus(GEO) is an open-access database managed under NCBI, which organises, stores and provides experimental data sets of genomics and gene expression experiments. It was launched in 2000 to provide a source for gene expression experiments data sets. By time it has developed with the technological advancements and now stores high-throughput data for different other applications like gene-protein interactions, chromatin methylation and structural analysis. GEO supports collaborative guidelines for few crucial study factors like different forms of data from all steps i.e from raw to meta [53]. Along with access to enormous experimental data, it also provides various web-based tools and algorithms that allows to filter data personalised according to the query demand and also its analysis and repersentation. Its homepage is available at http://www.ncbi.nlm.nih.gov/geo.

When we searched the GEO database for the dataset of cancer cell line treatment with ginger, we found a dataset of Series: GSE144235 in which MCF-7 cell lines were treated with ginger(https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE144235). From the raw dataset, we selected data of 10 Ginger treated MCF-7 Cell line samples (as Test group) and 10 DMSO treated MCF-7 cell line samples (as Control group) and then run analysis with GEO2R and in result data we got expression levels of 15 genes which are present in table 3.

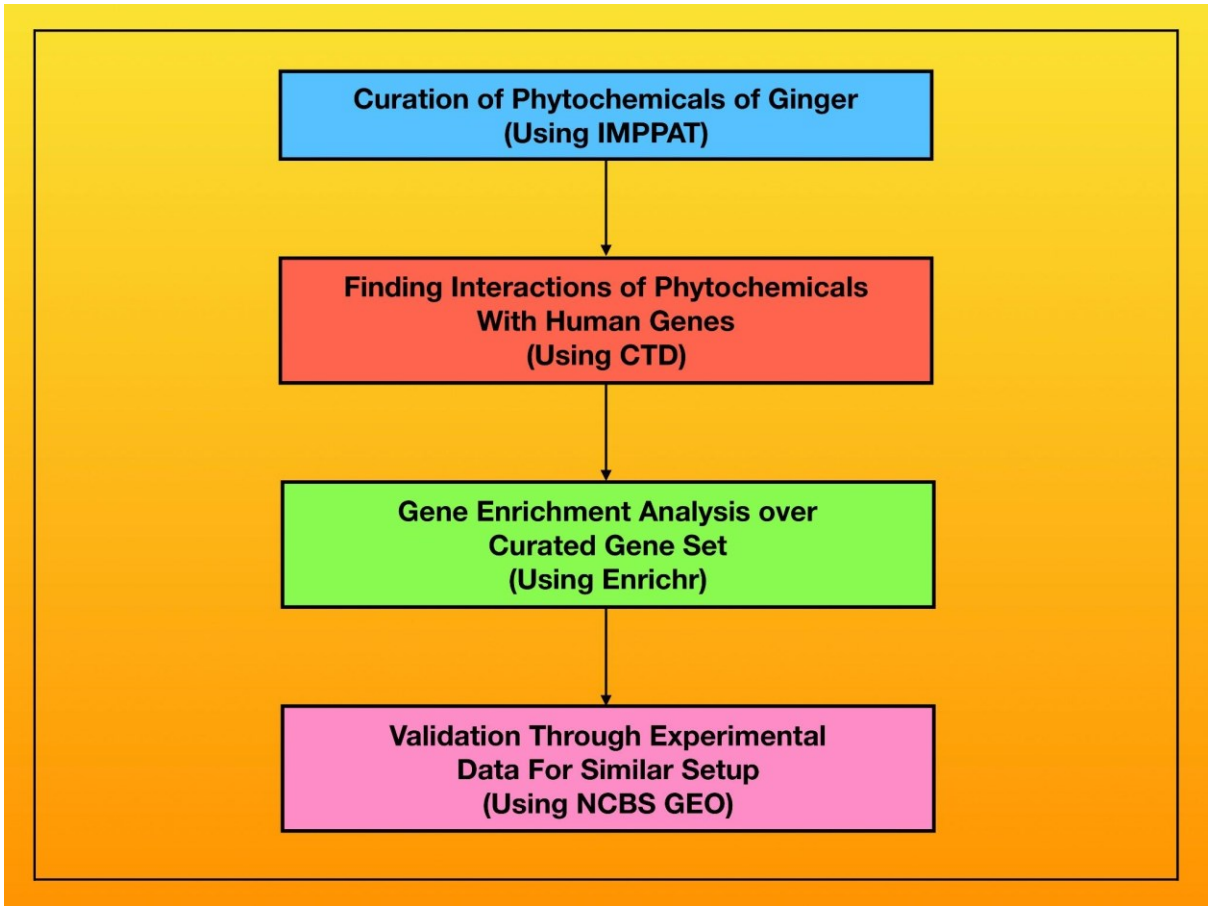

Figure 1 - Workflow of the methodology of the experiment.

CHAPTER-4 RESULT

After analysing the result from the Enrichr tool for our gene set(72 genes), we got major involvement of ginger phytochemicals in cancer pathways(32 genes) and apoptosis(22 genes), along with a list of genes involved in those processes. After cross-checking the expression level of those genes involved in cancer pathways in the experimental dataset obtained from GEO, we found around 11 genes are normally regulated - AKT1, BAX, BCL2, CASP3, CDK2, CDKN1B, IKBKB, KEAP1, NFE2L2, PPARG & PTGS2, and 4 genes are upregulated- CDKN1A, FAS, HMOX1 & NFKBIA.

1-Nonanol	L-cysteine	Gingerdione	Dipentene	6-Gingesulfonic acid
Angelicoidenol	l-isoleucine	Gingerenone A	DITERPENE II (LACTONE)	6-Shogaol
cis-Sesquisabinene hydrate	L-leucine	Gingerenone B	DL-Arginine	alpha-Farnesene
(-)-camphene	L-serine	Gingerenone C	DL-ASPARTIC ACID	alpha-TERPINEOL
(-)-Linalool	L-threonine	Gingerglycolipid A	DL-Valine	beta-Bisabolene
(?)-10-Epizonarene	L(-)-Borneol	Gingerglycolipid B	ent-Zingiberene	BETA-PHELLANDRENE
(+)-alpha-phellandrene	Methyl-[12]-Gingediol	gingerol	Eucalyptol	BETA-PINENE
(+)-Sabinene	MYRCENE	glycine	GAMMA-TERPINENE	beta-Sesquiphellandrene
(E)-.beta.-Farnesene	nerol	HEPTANE	GERANIOL	Citral
(S)-3alpha-[(S)-1,5-Dimethyl-4-hexenyl]-6-methylenecyclohexene	octane	Hexahydrocurcumin	[(2R,4R,5S,6R)-3,3,4,5-tetrahydroxy-2-propoxy-6-[[(2S,3S,4S,5R,6R)-3,4,5-trihydroxy-6-	Dehydro-10-gingerdione

			(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-4-yl] (Z)-octadec-9-enoate	
[4]-Gingerdiacetate	sesquithujene	Isogingerenone B	Gingerdiol	Dehydrozingerone
[6]-Gingerdiol 3,5-diacetate	Vanillylacetone	l-alpha-Curcumene	3-Methylbutanal	6-Gingerdiol
1-(4-Hydroxy-3-methoxyphenyl)-3,5-octanediol	1-Dehydro-6-gingerdione	1-(4-hydroxy-3-methoxyphenyl)tetradecane-3,5-diol	GERANYL ACETATE	

Table 1 - List of phytochemicals of Ginger curated from IMPPAT.

Gene Symbol	Gene ID	Gene Symbol	Gene ID
PARP1	142	**IL1B**	3553
AKT1	207	**MMP9**	4318
FAS	355	**NFE2L2**	4780
FASLG	356	**NFKB1**	4790
AR	367	**NFKBIA**	4792
BAX	581	**NGFR**	4804
BCL2	596	**NOS2**	4843
BCL2L1	598	**NPY1R**	4886
BDNF	627	**NPY2R**	4887
CASP3	836	**PPARA**	5465
CASP9	842	**PPARG**	5468
CDK2	1017	**MAPK1**	5594
CDKN1A	1026	**MAPK3**	5595
CDKN1B	1027	**PTGS2**	5743
CHAT	1103	**RELA**	5970
CHUK	1147	**SOD2**	6648
CTNNB1	1499	**SREBF2**	6721

CYP2B6	1555	**TNF**	7124
DDIT3	1649	**TRPV1**	7442
DFFA	1676	**TNFSF10**	8743
NQO1	1728	**CFLAR**	8837
ESR1	2099	**KAT2B**	8850
ESR2	2100	**ADIPOQ**	9370
F2	2147	**GDF15**	9518
FABP4	2167	**PTGES**	9536
FTL	2512	**KEAP1**	9817
GABRA1	2554	**NR1I3**	9970
GABRB2	2561	**MT1**	17748
GABRG2	2566	**SIRT3**	23410
GCLC	2729	**AKR1B10**	57016
GCLM	2730	**TRPV4**	59341
GSK3B	2932	**SLC5A7**	60482
H2AX	3014	**GGTLC1**	92086
HBB	3043	**OLFR73**	117004
HMOX1	3162	**OBP3**	259247
IKBKB	3551	**GOBP2**	693052

Table 2 - Set of genes with whom the phytochemicals of ginger bind currated using CTD database.

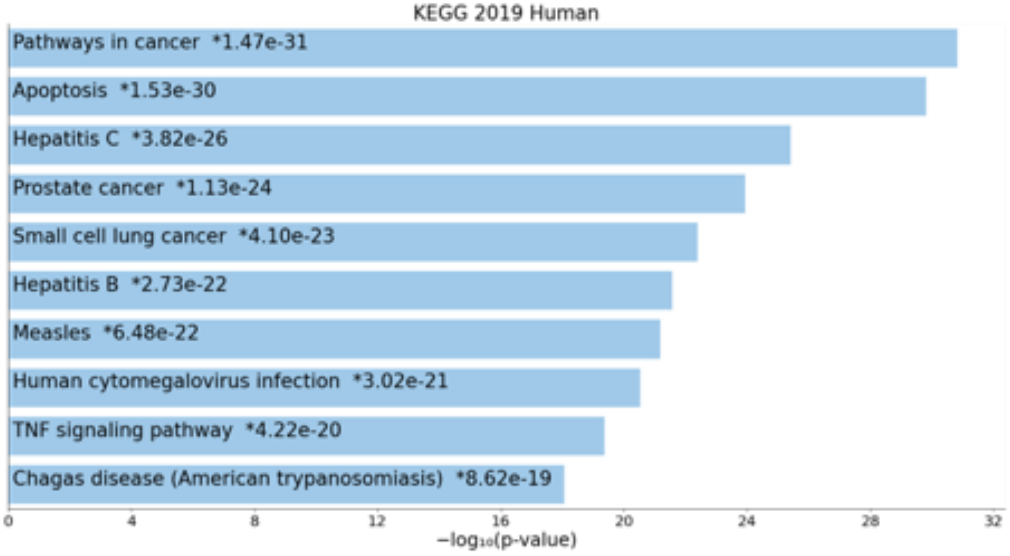

Figure 2 - Bar graph of result after gene enrichment of gene set of 72 genes using Enrichr.

NFE2L2	**ESR1**	**MAPK1**	**HMOX1**	**FASLG**
BCL2L1	**ESR2**	**MAPK3**	**AKT1**	**KEAP1**
GSK3B	**NFKB1**	**NQO1**	**CASP3**	**CDKN1B**
PPARG	**NFKBIA**	**CHUK**	**IKBKB**	**CDKN1A**
FAS	**AR**	**NOS2**	**CASP9**	
CTNNB1	**CDK2**	**F2**	**RELA**	
BAX	**BCL2**	**MMP9**	**PTGS2**	

Table 3 - List of 32 genes with whom the phytochemicals of ginger bind and are involved in pathways of cancer.

Gene symbol	ID	adj.P.Val	P.Value	t	B	logFC	SEQUENCE	SPOT_ID
AKT1	207163_s_at	0.832	0.703106	-3.87E-01	-5.6509	-3.33E-02	TAGCACTTGACCTTTTCGACGCTTAACCTTTCCGCTGTCG	207163_s_at
BAX	**208478_s_at**	0.832	0.697171	0.395	-5.6479	6.93E-02	GACCCGGTGCCTCAGGATGCGTCCACCAAGAAGCTGAGCG	208478_s_at
BCL2	**203685_at**	0.269	0.054387	-2.05	-3.9537	-5.47E-01	TTTCATTAAGTTTTTCCCTCCAAGGTAGAATTTGCAAGAG	203685_at
CASP3	**202763_at**	0.653	0.419851	-0.824	-5.4073	-1.4E-01	ACTGCACCAAGTCTCACTGGCTGTCAGTATGACATTTCAC	202763_at
CDK2	**204252_at**	0.541	0.301449	-1.06	-5.2069	-1.76E-01	TGATCCCATTTTCCTCTGACGTCCACCTCCTACCCCATAG	204252_at
CDKN1A	**202284_s_at**	0.115	0.002679	3.44	-1.4962	6.86E-01	CAGACATTTTAAGATGGTGGCAGTAGAGGCTATGGACAGG	202284_s_at

CDKN1B	**209112_at**	0.494	0.255035	-1.17	-5.0977	-9.86E-02	CCAAAGTGGCATGTTTTGTGCATTTGTAAATGCTGTGTTG	209112_at
FAS	**204781_s_at**	0.2	0.02169	2.5	-3.2172	6.26E-01	AGAAAGTAGCTTTGTGACATGTCATGAACCCATGTTTGCA	204781_s_at
HMOX1	**203665_at**	0.146	0.008296	2.94	-2.4301	1.94	AAGTATCCTTGTTGACACGGCCATGACCACTTTCCCCGTG	203665_at
IKBKB	**209341_s_at**	0.295	0.077511	-1.87	-4.23	-2.53E-01	TTTGTTGGAGAAGAAAGTTGGAGTAGGAGACTTTCACAAG	209341_s_at
KEAP1	**202417_at**	0.836	0.712857	0.374	-5.6557	2.96E-02	TACATAGAAGCCACCGGATGGCACTTCCCCACCGGATGGA	202417_at
NFE2L2	**201146_at**	0.776	0.610317	-0.518	-5.5957	-5.21E-02	CCTGCAGCAAACAAGAGATGGCAATGTTTTCCTTGTTCCC	201146_at
NFKBIA	**201502_s_at**	0.173	0.011122	2.81	-2.6713	4.39E-01	GCTCAGGAGCCCTGTAATGGCCGGACTGCCCTTCACCTCG	201502_s_at
PPARG	**208510_s_at**	0.669	0.440611	-0.787	-5.4343	-6.33E-02	TGCTCCAGAAAATGACAGACCTCAGACAGATTGTCACGGA	208510_s_at
PTGS2	**204748_at**	0.541	0.301519	1.06	-5.207	1.13E-01	GGGAATTTGGGTTGTGTATGCGAATGTTTCAGTGCCTCAG	204748_at

Table 4 - Gene expression of genes that are involved in pathways of cancer and phytochemicals of ginger bind with them when cell lines are treated with ginger. This is curated from microarray data obtained from NCBI GEO of MCF-7 cancer cell lines treated with ginger.

CHAPTER-5 DISCUSSION

CDKN1A possesses a critical role in regulating cell-cycle development and DNA damage-induced G2 arrest [54]. FAS-mediated apoptosis could play a role in peripheral tolerance induction, antigen-stimulated mature T-cell suicide, or both.[55]. Since an accumulation of free heme makes cells more susceptible to apoptosis, HMOX1 has cytoprotective properties. NFKBIA is actively involved in adaptive immunity pathways and after being phosphorylated promotes ubiquitination and degeneration, which allows relocation of the dimeric RELA from cytoplasm to the nucleus and further activation of transcription [56].

AKT phosphorylates GSK3 isoforms, which triggers cell proliferation. It also monitors cell viability by phosphorylating MAP3K5 which is a kinase related to apoptosis signal [57]. Phosphorylation of TSC2 at 'Ser-939' and ‘Thr-1462', by AKT, mediated insulin-stimulated protein synthesis activates mTORC1 signalling and leads to both activations of RPS6KB1 and in phosphorylation of 4E-BP1 [58]. AKT is also engaged in the phosphorylation of FOXO factor partners (members of the Forkhead transcription factor family), which results in the cytoplasmic localization and association of 14-3-3 proteins. [59]. In the coordination of gene transcription which is NF-kappa-B-dependent also, AKT plays an important role and constructively controls the function of CREB1 (cyclic AMP (cAMP)-response element-binding protein) [60]. The phosphorylation of CREB1 causes the aggregation of accessory proteins essential for the transcription of pro-survival genes like MCL1 and BCL2. [60].

Our study shows, genes like AKT1, BAX, BCL2 and CASP3 also function together to promote apoptosis while PTGS2, IKBKB, NFE2L2, KEAP1 and others are involved in inflammatory and other immune responses. Thus, we conclude that not only do the ginger components tested have anti-cancer activity but also possess immunomodulatory capabilities thus giving us a better understanding of the multi-faceted role of ginger and its potential mechanism of driving immuno-therapeutic strategies. Although polyphenols are well explored for their therapeutic role in many cancers, wet lab validation of ginger components may be further developed for devising a better combinatorial therapeutics.

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