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Herbal informatics: A unique model to identify the anti-cancerous agents for targeting lung cancer

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Highlights.

- *Lung cancer is a life threating disease, which causes 60% of deaths in patients within a year.*
- *Current management is not effective to ameliorate the progression of disease and has several side effects*
- *This study utilized a novel herbal informatic to provide an alternative curative drug using binary index and rationale-based selection.*
- *Study scrutinized 07 natural products, that has drug ability to target lung cancer in mammalian systems followed by its cross validation at molecular docking level.*
- *This unique bioprospection study could be beneficial for drug discovery process in future.*

Abstract

The incidence of lung cancer has increased in recent years and causes major mortalities across the globe . Besides, the availability of the several chemotherapeutics modalities in the management, there is still a challenge to find out the efficient remedy with lesser or no toxic effects. Hence, there is a necessity to employ a complementary research to establish the effective management for the lung cancer. In this study, we have implemented a novel herbal informatics model to find out the alternative remedy in treatment of lung cancer. This model utilizes five major steps of bioprospection process based on the classical surge followed by the binary index and rationale-based selection of herbal products targeting the cancer-causing factors which is explained in detail in the methodology section of this model. This study revealed 07 herbals such as *Withania somnifera (Ws), Berberis vulgaris(Bv), Glycyrrhiza glabra(Gg), Andrographis paniculate(Ap), Azadirachta indica(Ai), Cinnamomum verum(Cv), Piper longum(Pl)* based on the fuzzy set optimization scoring(0.6-1) that could be further studied *in vitro* and *in vivo* level for utilization in the management of lung cancer.

Keywords: Herbal Informatics; Ayurveda; Lung Cancer; Ethnopharmacology; Natural Compounds; Alternative Medicine doi:10.20944/preprints202012.0132.v1

1.Introduction

Lung cancer is a major health issue, that is caused due to a variety of genetic and environmental factors and is reported to cause the major mortalities all around the world **[1, 2]**. Researchers, while predicting the mortalities related to lung cancer globally, estimated around 1.7 million deaths in 2018 **[3]**. Two major categories of lung cancer, namely, Small Cell Lung Cancer (SCLC) and Non Small Cell Lung Cancer (NSCLC) account for 15% and 85% of all Lung cancer cases respectively. Histologically and clinically, NSCLC is categorized into adenocarcinomas, squamous cell carcinomas and large cell carcinomas **[4]**.

The various pathways like the tyrosine kinase family, epidermal growth factor receptors (EGFR) are over expressed in cancers. Various other anomalies have also been reported like, KRAS gene mutation, PI3K gene mutations, fusions in ALK genes etc.[5] Vascular endothelial growth factor (VEGF), that underlies angiogenesis is also reportedly increased in NSCLC and other cancers **[6].** The gene encoding for Anaplastic Lymphoma Kinase(ALK) is reported to undergo rearrangement in some cases of non-small cell lung cancer (NSCLC). **[7].** Anaplastic lymphoma kinase (ALK) and echinoderm microtubule-associated protein-like 4 (EML4) are over expressed in 3–13% of lung cancers.**[8, 9].** In hypoxic state Histone Deacetylases (HDAC) causes angiogenesis.**[10]**The phosphoinositide 3-kinase (**PI3K**) /downstream serine/threonine kinase (Akt)are found to be involved in anti-apoptotic signals in non-small cell lung cancers (NSCLC)**[11, 12].** Tubulins, the multifunctional proteins participate in chromosome separation during cell division, intracellular transport, cell shape modulation. The γ-tubulin in non-small cell lung cancer (NSCLC) is over expressed and also impacts patient survival at advanced stages of NSCLC **[13]** Fibroblast growth factor receptor (FGFR) is involved in regulation of cell proliferation, differentiation, shape and movement and causes angiogenesis**[14, 15].** FGF 9 factor, ligand of FGFR3, is over expressed in lung breast, prostate cancers .[16] Both autocrine and paracrine pathways simultaneously activate epidermal growth factor receptors (EGFR) to secrete epidermal growth factors molecules, which activate targeted signal transduction pathways and transfer cellular information from EGFR to the inner side of lung cells.[17] The epidermal growth factors promote lung metastasis through EGFR-receptor tyrosine kinases (RTK) by initiating and triggering signaling cascade in NSCLC and SCLC both.[18]

Several factors/pathways can be targeted by active constituents present in herbal compounds to regulate the signaling pathways. As today apart from surgery, radiation, systematic therapy like chemotherapy, targeted therapy, antibody treatment and angiogenesis inhibitors medication are also given to lung cancer patients . Various chemotherapeutics agents are also available for general and targeted therapy for lung cancer that is approved by FDA (The US Food and Drug Administration) and/or EMA (European Medicines Agency) such as carboplatin, cisplatin, paclitaxel, Gefitinib (anti-EGFR), Crizotinib (anti-MET, anti ALK and anti ROS1), Lorlatinib (anti-ALK) etc. [19]. The proliferating cancer cells are susceptible to chemotherapy but also has various side effects like nausea, diarrhea, weakness, lethargy, hair loss as routine. Some-times chemical drugs may have serious side effects like myelotoxicity [20], cardiotoxicity[21] , renal toxicity [22] Chemotherapy medicines may affect blood-forming cells in bone marrow. The long term usage of anti-cancer drugs is seen in increasing mutation in EGFR gene and amplifying mesenchymal–epithelial transition (*c-MET*) proto-oncogene and it also leads to the development of non-responsiveness to these therapies.[23]

Herbal compounds are most popular among all medications known till date, and has several medicinal properties like healing, immune boosting, anti-bacterial, rejuvenating and purifier activity in them. They are being used as primary medicines to treat number of disorders with less or no side effects.[24] In Ayurveda , thousands of years ago ,the herbals with their therapeutic uses were described to treat various human disorders as various forms of concoction. The NIH National cancer institute (NCI) reported 3000 herbals with confirmed anticancer properties [25]. Herbal derived anticancer medicines have been categorized into four classes according to their mechanism of action such as Tubulin inhibitors (vinca alkaloids, taxanes), HDAC inhibitors (various isoflavonoids), Topoisomerase I and II inhibitors (Camptothecins and podophyllotoxins) and DNA disrupters (epigallocatechin-3-gallate)[26]. Various herbals have been proven to exert anti-cancer mechanisms via regulating various pathways. Neem (*Azadirachta indica*), a well-known Ayurvedic herb, has been shown to exert various activities like antibacterial, antifungal, anti-inflammatory etc. It has also displayed anti-carcinogenic activities via phytochemicals like quercetin, nimbolide etc. [27, 28] In this research article, various other herbal compounds have been described in **Table 2,** with similar properties. These herbals have a number of active ingredients, but these molecules and their target hits have to be identified. The various side effects of chemotherapy in patients has persuaded scientist to study herbals derivatives as alternate medicines to alleviate disease like cancer. The complex nature of lung cancer poses challenges to present medicines available and persuades researchers to find new medicines. The present insufficient systematic research for herbals and their specific molecule is a limiting factor for lung cancer treatment. The traditional process is tedious and time consuming and thereby irrational. In this study *in-silico* herbal informatic model, we are bio-prospecting the several medicinal herbs to identify their potential for targeting specific factors/pathways that are responsible for lung cancer. Web based data search engine, matrix linked data mining and Fuzzy score based validation and identification for probable therapeutic efficiency of the screened plants have been done. The large analogous research on target factors of lung cancer and herbs are available on web, which were filtered on number of direct hits for further '*in silico'* research. This novel tool thus widens the opportunity for further *'in vivo'* or *'in vitro'* research and systematic development of lung cancer targeting plant-based drugs based on the plants shortlisted. [29].

2. Methodology

2.1 Traditional literature surge model

The WHO data for lung cancer estimated 2.09 million cases and 1.76 million mortalities in 2018. American Cancer Society estimated 135,720 deaths from lung cancer (72,500 in men and 63,220 in women) in USA in 2020 **[30].** The present limited treatments for lung cancer, chemotherapy limitations and its side effects with poor recovery rates (According to latest lung cancer statistics, five year survival rate is 19%) are the basis for selecting lung cancer for herbal targeting. A classical literature torrent search was done to identify 10 specific pathological factors like Tubulin ,Histone Deacetylases (HDAC), Epidermal Growth Factor Receptor (EGFR), Anapestic Lymphoma Kinase (ALK), Fibroblast Growth Factor Receptor (FGFR) and Phosphoinositide 3-Kinase (PI3K) , Akt (serine/threonine-specific protein Kinase B), Signal Transducer and activator of transcription 3(STAT3) and KRAS (Kirsten rat sarcoma viral oncogene homolog) were selected as presented in **Table 1.**

Similarly, database of herbals was prepared based on the selection criterions included Ethno-pharmacological properties, Traditional uses, Availability at ease, therapeutic potential associated with indirect indications and literature support as exemplified in **Table 2** [31].

S. No	Bioactivity parameters (BAP)	Rationale for selection (Based on classical surge)
	EGFR	i. Growth factor receptor (transmembrane) that is a part of HER/erbB tyrosine kinase family, and it controls proliferation and death of cell (apoptosis). This receptor is overexpressed in NSCLC. ii. According to research, Cucurbitacin B, a phytochemical, caused degradation of EGFR via lysosomes in NSCLC cells. iii. Plant product lupeol can cause reduction in EGFR protein by binding to it and phosphorylating it.
	ALK	i. Function as a receptor tyrosine kinase and it undergoes mutations (translocation/fusion) in various cancers increases proliferation, metastasis and prevents cell death. It plays an important role in development of lung cancer, mainly NSCLC. ii. Phytochemicals from *Vitex negundo* namely: 6'-para-hydroxybenzoyl mussaenosidic acid, protocatechuic acid, viridiflorol and betulinic acid could effectively inactivate ALK protein in lymphoma cells.
	PI3K (Phosphoinositide 3-kinase)	i. It is a kinase enzyme that acts in converting PI (3,4)-bisphosphate to PI (3, 4, 5)-trisphosphate leading to subsequent localization of Protein Kinase B (Akt) to the cell membrane. In various cancers, this kinase enzyme is upregulated (over-expressed) and is crucial in apoptosis, metastasis, angiogenesis etc. ii. Involved in lung cancer and it frequently was found to co-exist with deletion (loss) of PTEN genes, iii. Phytochemicals (e.g. Andrographolide from *Andrographis 5tilbene5e*) that cause inhibition of PI3K or the downregulation of its phosphorylated forms and these can be beneficial in cancer therapy.
	Tubulin	i. Important structural components of microtubule and these exist in the form of two subunits namely alpha and beta. These are involved normally in transport within the cell, signaling of the cell and various stress responses. ii. Responsible for development of aggressiveness and resistance to therapy in various cancers and involved in the progression of adenocarcinoma of lungs and in conferring chemo-resistance to the tumors. iii. Various compounds from plant *Vinca* (Vincristine, Vinblastine) can block the assembly of tubulins.

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HDAC (Histone Deacetylase)	i. Protein that is involved in the deacetylation reaction in the histones of chromatin but in case of many cancers the activity of this protein becomes over expressed and the resulting reduced acetylation of histones causes faulty expression of various genes. ii. According to research, inhibitors of HDAC (namely SAHA) can reduce the growth of NSCLC tumors and it's also linked HDAC to SCLC by showing that an inhibitor of this protein (namely TSA) can reduce the proliferative properties of SCLC cells. iii. Phytochemicals like curcumin can cause the reduction in the expression of various types of HDAC in various cancer cell lines.
Fibroblast growth factor receptor (FGFR)	i. It is a type of receptor tyrosine kinase present in cell membrane that normally in involved in growth processes, survival, and differentiation of cells. ii. In many cancer cell types; research has shown this receptor to be over expressed and significant proportion of patients with NSCLC had elevated levels of FGFR and its also demonstrated that the inhibition of this protein led to reduction in growth and proliferation of the tumor cells. iii. Various phytochemicals show anti-FGFR activities e.g. *Boswellia papyriferaii* derived 6 tilbene glycosides directly block FGFR by binding to it.
Serine/threonine-specific protein Kinase B(Akt)	i. It is also called Protein Kinase B and is involved in survival of the cells. ii. It is reported to be activated by various growth factors and is found to be over-expressed in many cancer types and significant proportion of NSCLC cells this protein is overexpressed, and it causes the cells to acquire immunity to various therapies (Chemo and radiotherapy). This protein is reported to cause delaying of apoptosis in the NSCLC cells. iii. Study reported that a naturally occurring phytochemical, Piperine, causes blocking of Akt protein and thus it has anti-angiogenesis properties against various cancer cells.
STAT3 (Signal Transducer and Activator of Transcription 3)	i. It is an important component of the signaling pathway in cytoplasm and it also acts as a factor of transcription. ii. Researchers have reported that in various cancers, this factor is involved in conferring malignancy and chemoresistance to cells as well as enhancing their proliferation. STAT3 has also been found to be involved in lung cancer, especially NSCLC where it is over-expressed (in both phosphorylated and normal forms). iii. Study reported that phytochemicals could inhibit the phosphorylation of STAT 3 by TNF-alpha and blocked its translocation to the nucleus and could effectively block phosphorylated STAT3.
KRAS	i. It is a member of RAS family that encodes two GTPase proteins namely KRAS4A and KRAS4B as it undergoes alternative splicing. It has been reported that in NSCLC, KRAS gene is mutated and it leads to development of tumor. ii. Researchers reported that mutations in RAS is cause over activation of RAF-ERK and PI3k-Akt pathways. iii. Phytochemical Krukovine, obtained from *Abuta grandifolia* caused reduction in growth of Lung cancer cells with mutated KRAS as its inactivated RAF-ERK and Akt pathways.

Bcl-2 (B-cell Lymphoma 2)	i.	Family of proteins that plays important role in apoptosis and it causes prevention of programmed death of the cells.
	ii.	The expression of BCL-2 and thus the subsequent delay in apoptosis has been observed in both SCLC and NSCLC.
	iii.	Research showed that phytochemical from *Mentha arvensis*, could cause down regulation of BCL-2 and this can also be used for its anti-cancer property.

Table 1- Bioactivity parameters and their reason for selection

2.2 Relevance factors-based analysis of binary matrix

50 herbals were identified initially as anti-cancer agents. The combination keyword search combining Anti-cancer agents and lung cancer causing factor inhibition, followed by linking it with observations based analysis of the first 20 hits, relevance factor/Net-Weightage of each cancer causing factor was evaluated using following formula **(Equation 1)**

$$Percentage\ relevance = \frac{Relevant\ Hits \times 100}{20}$$

… (Eqs1)

The primary database set of Herbal Plants combined with the presence/absence of a given parameter was prepared using the web search engine, PubMed (N= first 20 hits) with a median value as cut off filter value. **[32]**

2.3 Relevance factor linked Binary weightage matrix-based analysis

The relevance of each cancer-causing factor was used for calculation of its respective Weightage score and subsequent analysis by consideration of every plant with respect to all the cancer causing factors taken together was performed. These parameters for weightage calculation increased the 'uncertainty factor' required for statistical analysis to be viable and bring about reduction in "Investigator's prejudice". Herbals with binary score ≥10, from previous step were taken into consideration for further study.

2.4 Fuzzy set membership analysis for decision matrix & optimization

The mathematical relationship as mentioned below **(Equation. 2)** was used to assign the relative relevance via Fuzzy score analysis within identified group of plants.

$$\mu S = Score - MinS / MaxS - MinS$$

… (Eqs2)

Where, μS represents the desirability values of selected herbal plants of the fuzzy set S. Min (S) and Max (S) correspond to the minimum and maximum fuzzy scores, respectively, in the fuzzy set under consideration (S). The estimated desirability values (μS) were converted into a leveled score via a scaled magnitude to optimize the values and thus leading to identification of potential anti-lung cancer herbal agents. **[33]**

3. Results

3.1 Classical literature surge:

The classical literature search brought forward 10 cancer causing factors as shown in **Table 1** and the data base of 50 plants eventually led to shortlisting of **19 herbal compounds** whose Binary score value range from 4 to 14 with mean value 8.85) and weightage score ranging from 39.789 to 222.156 (mean value = 133.167) which showed relevance towards the 10 pathological factors are described in **Table 2**.

S.No.	Herbals	Herbal code	Region	Active phytoconstituents	Ethno-pharmacological importance	Traditional use
1.	*Andrographis paniculata* *Hindi: Kirayat* *English: King of Bitters*	Ap	India Malaysia South east Asia	Andrographolide (**Anticancer**), Neoandrographolide, Dehydroandro grapholide	**Antitumor** Anti-inflammatory, Immune booster, Analgesic, Antiviral Cardioprotective, Hepatoprotective	Tonsillitis, Tuberculosis, Pneumonia, Hepatitis A, Leptospirosis
2.	*Azadirachta indica* Hindi: Neem English: Azadirachta Indica	Azi	India Asia		**Anticancer** **Antitumor,** Antioxidant, Immunostimulatory, Hepatoprotective	skin disorders, pains, fever, antibacterial Antiviral Insecticide. Used in dengue
3.	*Berberis vulgaris* Hindi: Daruharidra, Daruhaldi English: Barberry	Bvi	India, Iran, Italy, Bulgaria, China, Turkey France,	**Berberine, Berbamine,** Acanthine Aesculetine, Berberrubine, Chrysanthemice, Columbamine,	**Anti-cancer, Antitumor,** Anti-inflammatory, Antioxidants, Cardioprotective, Neuroprotective, Anti-arthritis	kidney diseases, hypertension, liver diseases, Respiratory diseases
4.	*Caesalpinia sappan* Hindi: Patranga English: Brazil wood	Cs	India, China, Japan, Brazil, South East Asia, Europe	**Brazillin** Caesalpin J ,P Protosappanins, Ombuin, Rhamnetin, Chromanones, Chalcones	Anticancer, Antitumor, Immune booster Anti-inflammatory, Anticoagulant, Hepatoprotective	Used for wounds healing, ulcers, leprosy, epilepsy, diabetes, rheumatism.
5.	*Capparis spinosa* Hindi /Sanskrit: Kantaka English: Caper bush	Csp	India Mediterranea n region, Africa, West and Central Asia	Quercetin glucocapparin, glucoiberin, glucopangulin, astragalin, Cappariside, corchoionoside	Anti-cancer, Antitumor, Anti-inflammatory, Antioxidant, Hepatoprotective Anti-HIV, Antimutagenic	Used in diarrhea, gout, rheumatism, spleen diseases, eye diseases.

6.	*Cassia tora* Hindi: Charota English: Sickle Senna	Ct	India, Sri Lanka, West China	modin, Chrsyphanol, anthraquinone, Rubrofusarin, Friendlin, Quercetin, Aurantio-obtusin Obtusifolin,	Anticancer, Antitumor Antioxidant, Anti-inflammatory, Antihepatotoxic, Antimutagenic,	Used in liver disorders, blurriness of eyes, bronchitis, cough, heart disorders,
7.	*Cinnamomum verum* Hindi: daalacheenee English: Cassia	Cv	Sri Lanka and Southern parts of India	2 Methoxycinnamaldehyde, Cuminaldehyde, Gallic acid,	**Anti-lung cancer** Antioxidant, Antimicrobial, Antifungal, Hypotensive,	Antimicrobial used in Respiratory, digestive, cardiovascular, Alzheimer's disease,
8.	*Commiphora myrrha* Hindi: Bolah English: Myrrha	Cm	India Pakistan, Arabia, Southern Africa	**Guggulsterone** Eugenol, Myrrholic acid, lindestrene, myrrhone, anthracene Germacrene B	**Anti-cancer** Analgesic, Antibacterial, Antioxidant	Used in stomach illnesses, bronchitis, asthma, flu,
9.	*Foeniculum vulgare* Hindi: Saunf English: Fennel	Fv	India (mainly Northern India) Europe	Anethole Fenchone, Anisaldehye, Quercetin, Orutinoside, Rosmarinic acid	**Anticancer** **Antioxidant** Antibacterial, Anti-inflammatory, Anti-atherogenic, Hepatoprotective, Memory-enhancer.	Abdominal problems, sedative, anti-diabetes, bronchitis, kidney stones, eye diseases.
10.	*Glycyrrhiza glabra* Hindi: Madhuka English: Liquorice	Gg	India Temperate countries of the World	Formononetin Glycyrrhizin, Liquirtin, Liquirtigenin, licoriphenone,	**Anti-lung cancer** Anti-tumor, Antioxidant, Hepatoprotective, Anti-inflammatory, Immunostimulatory, Cardioprotective,	Used for cardiac diseases, haemorhage, eye diseases, jaundice. Osteoarthritis,
11.	*Hygrophila spinosa T* ander Hindi: Talim Khana English: Hygrophila,	Hsn	India (Himalayas) Sri Lanka, Nepal, Myanmar, Malay	Tannins, Lupeol, Betulin, Terpenoids, Alkaloids, Phenolic compounds,	**Anticancer, antitumor** Anti-inflammatory, Hematopoietic, Antioxidant Hepatoprotective, Analgesic,	Blood disorders, Biliousness, Hepatic obstruction, inflammations, joint pains,
12.	*Mentha arvensis* Hindi: Pudina (English): [Marsh mint	Ma	India Eastern Himalayas, Euresia, North and central America	Rosmarinic acid, Limonene, Luteolin, Caryophyllene, Neomenthol, Phellandrene, Hesperidin,	Anticancer, Antimicrobial, Insecticide Antifungal, Analgesic, Carminative,	Digestive disorders anti helminthic, cardio tonic pain reliever jaundice diabetes,
13.	*Morinda citrifolia*	Mc	India,	**Damnacanthal,**	**Anticancer, Antitumor**	diabetes, arthritis, pain,

	Hindi: Indian Mulberry, English: canary-wood		Southeast Asia, Australia, Polynesia,	**Citrifolinoside,** Scopoletin, Rubiadin, Morindone,	Antibacterial, Anthelminthic, Antituberculosis, Hypotensive, Immunostimulatory,	eye problems, hypertension, digestive problems, AIDS, depression,
14.	*Nigella sativa* English: Black cumin Hindi: Kalonji	Ns	India, Pakistan, Middle east and South East Asia	**Thymoquinone,** Cymene, Carvacrol, **Carvone, Nigellone** Nigellicine, Nigellidine, Hederin,	Anticancer Immuno booster Anti-inflammatory Antioxidant Analgesic, Anti-bacterial, Bronchodilator, Cardioprotective,	Diuretic, Diaphoretic, liver disorders, anti-obesity agent. leprosy, leukoderma,
15.	*Piper longum* Hindi: Pippali English: Balinese pepper	Pi	North-East India, Southern Sri Lanka	Piperine, Piperidine, Methyl piperine, pipenonaline, piperettine,	Anticancer, Antioxidant Anti-inflammatory, Hepatoprotective, Immunomodulatory, Analgesic Cardioprotective	Abdominal tumors and distention, respiratory disorders, laryngitis, epilepsy, gout, paralysis,
16.	*Urtica dioica* Hindi: Bichchhu *English: Stinging nettle*	**Ud**	India, Found Across the globe except all around Africa and Madagascar	Flavonoids, Polyphenols, Caumarins,	**Anticancer** Immunostimulatory, Analgesic Antioxidant, Anti-inflammatory, Neuroprotective	**Inhibit cell proliferation in Benign Prostatic Hyperplasia** allergies, diabetes, kidney stones,
17.	*Vinca rosea* Hindi: Sadabahar English: Cape periwinkle	Vr	India, West Indies, Nigeria	**Vinblastine, Vincristine, Vinorelbine** Vindesine Catharine,	**Anti-cancer Anti-tumor** Anti-diabetic Hypoglycemic	High blood pressure, diabetes infections
18.	*Withania somnifera* Hindi: Ashwagandha English: Indian ginseng	Ws	India (Western India, Bengal, Bombay), Africa, Mediterranean	Withanolides, Withaferin A, Wiithasomniferin-A,	Anticancer, Antitumor, Antioxidant, Neuroprotective, Antianxiety, Immunomodulator, Hepatoprotective	antioxidant, aphrodisiac, Liver tonic, insomnia memory loss, rheumatism, paralysis, immune system disorders, skin disorders

19.	*Wrightia tinctoria* English: Dyer's Oleander Hindi: *Indrajau*	Wt	Throughout India	Wrightial, Cycloartenone, desmosterol Cycloeucalenol, Gallic acid, Rutin, Quercetin	**Anti-cancer,** Anti-microbial, Anti-diabetic, Anti-ulcer, Anti-fungal, Antioxidant, Anti-helminthic, Anti-inflammatory	GI tract disorders., anti-venom (snake venom). skin diseases, inflammation, mumps, wound heal

Table 2- Ethnopharmacological properties of herbal plants showing direct/indirect indication for targeting lung cancer

3.2 Relevance factors analysis

Classical literature surge analysis was used to estimate the % relevance of each of the 10 cancer causing factors via the use of scoring analysis with keyword hits. Highest percentage relevance was obtained for Tubulin inhibition (95%) followed by ALK(90%), EGFR(90%) , HDAC(90%),FGER(85%),PI3K(85%), Akt(85%) , Bcl2 (80%) ,STAT 3(80%) and KRAS(75%) **(Table 3 & Figure 1).**

S.No.	**Bioactivity parameter (BAP)**	**Total no. of Hits**	**Hits relevance (N=20)**	**% relevance**	**Weightage score**
1	Tubulin	9825	19	95	21.00
2	Anaplastic lymphoma kinase (Alk)	2233	18	90	19.89
3	Epidermal growth factor receptor (EFGR	18770	18	90	19.89
4	Histone deacetylases (HDAC)	5678	18	90	19.89
5	Fibroblast growth factor receptor (FGFR)	1580	17	85	18.79
6	Phosphoinositide 3-Kinase (PI3K)	19479	17	85	18.79
7	Serine/threonine-specific protein Kinase 0B(Akt)	27087	17	85	18.79
8	B-cell lymphoma 2 (Bcl-2)	18379	16	80	17.684
9	Signal Transducer and activator of transcription 3(STAT3)	6116	16	80	17.684
10	Kirsten rat sarcoma viral oncogene homolog (KRAS)	3311	15	75	16.579

Table 3: Classical literature surge of Factors involved in lung cancer, showing relative percentage relevance

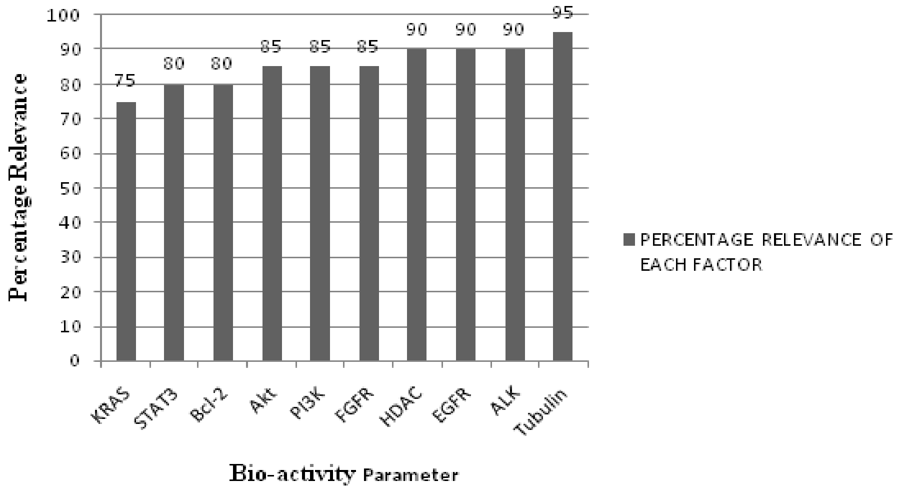

Figure 1: Percentage (%) relevance of Bio-Activity Parameter involved in the pathogenesis of Lung Cancer (KRAS= Kirsten rat sarcoma viral oncogene homolog, STAT3= Signal Transducer and activator of transcription, Bcl-2 = B-cell lymphoma 2 , Akt= Serine/threonine-specific protein Kinase B, PI3K = Phosphoinositide 3-Kinase, FGFR = Fibroblast growth factor receptor (FGFR), HDAC = Histone deacetylases, EGFR = Epidermal growth factor receptor, ALK = Anaplastic lymphoma kinase)

3.3 Relevance factor linked binary-weightage matrix-based analysis

The binary matrix analysis of presence/absence of the considered factors in 50 herbals revealed that 20 herbals showed binary score from 10 to 14. The consequent weightage score matrix analysis of 20 herbals revealed that seven herbals are having more than 10 and these are selected for present study.

3.4 Decision matrix-based Optimization

Based on decision matrix analysis, 7 herbals showed highest fuzzy score range from 0.6-1. Amongst these, *Withania somnifera* (score=1) held the topmost position with µS score being 1, relative to the lowest µS score exhibited by *Piper longum* (score=0.6). Optimized scores were also obtained for the selected herbals based on fuzzy set membership analysis by giving priority to highest score plant. These plants are *Withania somnifera (Ws), Berberis vulgaris (Bv), Glycyrrhiza glabra (Gg), Andrographis paniculate (Ap), Azadirachta indica (Ai), Cinnamomum verum (Cv), Piper longum (Pl)* as mentioned in **Figure 2**; **Table 4.**

S. No	Herbals	Herbal code	Binary Matrix Score	Fuzzy Score (µS)
1	*Withania somnifera*	Ws	14	1
2	*Berberis vulgaris*	Bvl	14	0.9533
3	*Glycyrrhiza glabra*	Gg	14	0.9467
4	*Andrographis paniculata*	Apn	14	0.9274
5	*Azadirachta indica*	Azi	13	0.8704
6	*Cinnamomum verum*	Cv	11	0.7461
7	*Piper longum*	Pl	10	0.6010

Table 4: Fuzzy set membership analysis for herbal plants screened based on binary and weightage matrix scores.

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4. Discussion

The chemotherapy kills the proliferating cancer cells but their long-term uses may cause other serious pathologies like myelotoxicity [20], cardiotoxicity [21], renal toxicity [22], mutation in EGFR gene to the patients [23]. As evident from large number of mortalities because of lung cancer every year or with poor recovery rates the chemotherapy alone is not able to control lung cancer completely . According to the latest lung cancer statistics, the five year survival rate is 19% which has suggested, there is still a need to find alternative medicines with the holistic approach in treatment. Herbal compounds have various medicinal properties like anti-oxidant , anti-inflammatory, immune booster, antiviral, antibacterial properties etc. as described in **Table 2** and number of herbals have shown anti-tumor activities also like *Withania somnifera,, Cinammomum verum, Nigella sativa, Vinca rosea.*

In this present study '*in silico'* bioprospection analysis is done, which includes using of PubMed as a random search engine for identification of the potential herbal agents based on rationale selection. Relevant factors (Bio-activity parameters) were selected with respect to their involvement (both direct and indirect) in the pathogenesis of lung cancer. The binary matrix approach is used to identify the herbal agents in this paper on the basis of fuzzy score analysis and these herbals have been shortlisted on the basis of all or none principle, with inclusion of herbal agents only with binary score ≥10 out of 14. After the first screening step, out of 50 herbals, 20 herbals were screened out for performing weightage matrix and the fuzzy set membership analysis, which finally provided a database of 07 herbals (Figure 2) that showed an acceptable optimized score such as *Withania somnifera(Ws), Berberis vulgaris(Bv), Glycyrrhiza glabra(Gg), Andrographis paniculate(Ap), Azadirachta indica(Ai), Cinnamomum verum(Cv), Piper longum(Pl).*

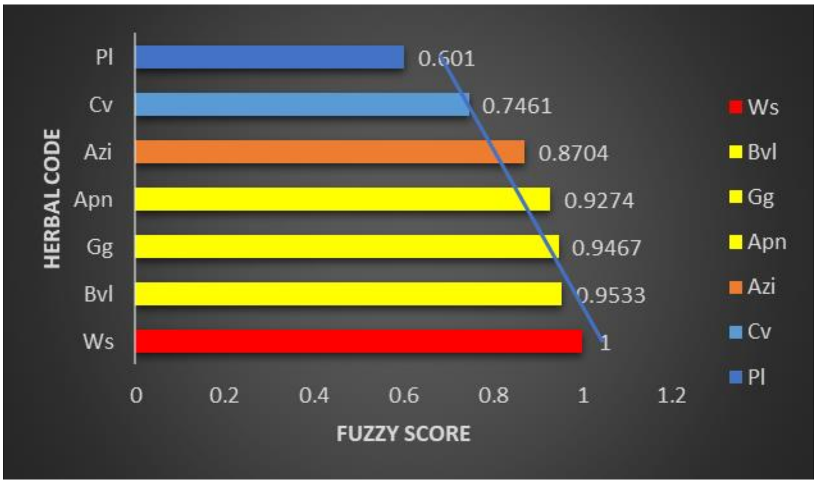

Figure 2: Fuzzy set Optimization of scrutinized Herbals

These scrutinized herbal compounds and their active constituents could also be studied in Molecular Docking and also at *'in vitro'* and *'in vivo'* model to further assessment of their pharmacological properties in laboratory and their anti- cancer potential can be further tapped in alleviating the disease for drug discovery process.

Conclusion

Herbal informatic study has provided 07 Herbal compounds i.e., *Withania somnifera(Ws), Berberis vulgaris(Bv), Glycyrrhiza glabra(Gg), Andrographis paniculate(Ap), Azadirachta indica(Ai), Cinnamomum verum(Cv), Piper longum(Pl)* with significant therapeutic potential targeting several disease factor of lung cancers. This study needs to be cross validated at docking level and at in *vitro* and *in vivo* level to further establish their role in management of cancer

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Conflict of interest

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