Print ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Pharmaco-Toxicological Evaluation of a Novel Anticancer Polyherbal Formulation Using *In Silico* Method: PASS

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ABSTRACT

The aim of the present study is to predict the pharmacological and toxicological activities of a novel polyherbal formulation (PHF) and also to verify its anticancer properties using an *In Silico* tool called PASS (Prediction of Activity Spectra for biologically active Substances) by which we can predict the activities theoretically. The PASS software which has a database predicts about 3750 pharmacological and 2000 toxicological activities based on its structural activity relationship. The predominant compounds present in the PHF were determined using GC-MS (Gas Chromatography – Mass spectrometry). In our study we have used this software to predict the anticancer property of the polyherbal formulation and found that it has potential to act as an anticancer agent with minimal side effects. Further *in vitro* and *in vivo* studies are required to validate these results.

KEY WORDS: Polyherbal formulation, Anticancer, PASS, *in silico.*

1. INTRODUCTION

Cancer is one of the predominant diseases worldwide characterised by genetic alteration leading to uncontrollable cell proliferation. The treatments available today are expensive and mediocre which bring about deleterious side effects. Hence efforts should be undertaken to find a suitable anticancer drug. Herbs contain naturally active secondary metabolites which gives them nutritional value. These compounds help in maintaining the energy balance in our body. These natural products due to their biological origin have better ADME (Absorption, Distribution, Metabolism and Excretion) and hence there is an enhanced tolerance in our body for this compound (Goel, 2010). These active principles present in the herbs have medicinal value with lesser toxicity when compared to the chemically synthesized compounds, so it is imperative to find an anticancer drug of plant origin with minimum or no side effects.

While considering herbal formulation for treatment of cancer it is important to understand that each herb has its own unique character which helps in treating and controlling the disease. Further herbs as a combination can be used to effectively treat various types of cancer. These Polyherbal formulations (PHF) which are organic products have been used in treatment of diseases since early times as these formulations were easily available biological ingredients. A new combination of herbs have been derived to formulate this novel PHF for treatment of colon cancer made from the following herbs namely *Cissus quadrangularis* Linn. It is a perennial plant belonging to the family Vitaceae having antioxidant activity, used for treating gastritis, bone fracture healing, piles etc. This plant is known to have anticancer property along with its usage in treating of burns and wounds (Dwivedi, 2013). *Andrographis paniculata* (Burm f.) wall ex Nees is a herbaceous plant belonging to the family Acanthaceae. The leaves and roots of this plant is reported to have hepatoprotective potential, antioxidant, anti-inflammatory activity, blood purification, treatment of gastroenterititis and also have long since known to have effective anticancer potential (Kumar, 2004; Akbar, 2011). *Trichopus zeylanicus* Gaert a small herbaceous plant which belongs to the family Trichopodaceae has antioxidant activity and is used in cancer treatment (Tharakan, 2005). The extracts from these herbs are in the ratio of 2:1:2 in the novel PHF being studied for treatment of colon cancer. Preliminary studies to evaluate the effectiveness of the PHF are done using *In Silico* method (PASS).

India is known for its early usage of natural products in treatment of diseases and the culture is rich in traditional Indian medicines which were effectively used and was known as Ayurvedic medicines and have been in existence for more than a thousand years (Jamkhande and Barde, 2014). To better understand the pharmacological behaviour and the particular significance of the interaction of the plant compounds, virtual screening may be used (Rollinger, 2008). One such method is the Prediction of Activity Spectra for Substances (PASS) which is an *In Silico* method to determine the biological activity spectrum of a compound. This technique is based on the structural activity relationship of a compound and its biological activity. The activity of the given compound is found by comparing its structure with the structure of an already existing biologically active compound present in the database. This PASS prediction tool will predict the Pa: Pi (Active: Inactive) ratio at a prediction threshold of Pa>30%, Pa>50% and Pa>70%. When Pa>70% the substance has higher chances of exhibiting the activity in the experiment, whereas if the Pa<50% the substance is unlikely to exhibit the activity in the experiment. The recent version of PASS can predict 3750 pharmacological effects, mechanism of action and toxicity of the given compound with 95% accuracy. The effectiveness of the compound can further be verified using *in vitro* as well as *in vivo* assays.

2. MATERIALS AND METHODS

Through GC-MS analysis the bioactive compounds which were predominantly present in the PHF were identified as following Benzofuran-2-one, 4-amino-2,3-dihydro- , 8-Octadecenoic acid, methyl ester, stigmastanol, dodecanoic acid 10-methyl- methyl ester and octadecanoic acid, 3-hydroxy-2-(1-oxotetradecyl), methyl ester.

PHF: The PHF prepared by Lanson biotech, India using solvent free extraction method, is based on the plant combination given by us.

PASS online prediction: The web based application PASS predicts a number of Pharmacological and toxicological activities of a compound simultaneously. These predicted biological activities are in some way related to the physico-chemical characteristics of the compound hence the predicted activity of spectrum is known as the intrinsic property of the compound. It predicts the activity spectrum as a ratio of probable activity and probable inactivity. The Pa and Pi value ranges from 0.000 to 1.000. For prediction of the activity spectra using PASS software the SMILES (simplified molecular - input line - entry system) format or the MOL file format must be known (Parasuraman, 2015). The SMILES format was obtained from chemspider website. The obtained SMILES format was fed into the PASS online software for prediction of the biological activity spectra of the compound. When Pa>0.7 it is highly possible that the predicted activity will be seen while doing the experiment, but the compound might be an already present compound. When Pa<0.5 then the chances of finding the predicted activity occurring while doing the experiment is less but it may hint at finding a novel compound. PASS software which predicts the whole biological spectra of a molecule detects Pharmacological as well as toxicological effects. A compound can cause toxicity in an organism either by acting on one target or by acting on multiple targets thus making it difficult to evaluate the toxic profile of a compound (Poroikov, 2007). However PASS makes it easy to predict the toxicity of the given compound. Toxicity prediction is similar to that of the pharmacological activity prediction. In our study PASS was used as a preliminary tool to verify the activity of the PHF as an anticancer agent and also the possible toxic effects, so biological activities with Pa>0.7 were only taken into consideration.

3. RESULTS AND DISCUSSION

The predicted biological and toxicological activities of the above mentioned five compounds are given in the form of tables.1-5. Herbal formulations containing single herbs are much easier to study than studying PHF because when two or three herbs are combined to form a combination these herbs react with one another and act on multiple targets, produce synergistic effects, potentiates one another or act as antagonists to one another thus enhancing their biological activities as well as reducing the side effects. When a PHF is prepared we should bear in mind it might or might not cure the disease for which it is prepared but can be effective against some other disease. In our study PASS software was used to find the possible pharmacological and toxicological activities of the novel anticancer PHF. The Five compounds which were predominantly present in PHF showed anticancer activities. The three plants present in this PHF are shown to have anticancer properties individually, but their effectiveness as a combination is yet to be studied. Our aim was to evaluate their effectiveness using PASS as a preliminary tool in predicting the probable activities of the PHF (Iqbal, 2015). The results predicted showed that these compounds had the potential to curb cancer by direct as well as indirect mechanisms. The toxicity profile was also predicted using PASS and the common side effects which were repeatedly predicted were reproductive dysfunction, skin irritation and ocular toxicity. The other side effects predicted were much milder. Here it is relevant to mention the toxicity totally depends on the dose of the compound administered which cannot be predicted by PASS, hence the toxic side effect predicted may or may not manifest themselves while administering a therapeutic dose. Further it being a herbal formulation, our body may have enhanced tolerance to the drug and only very high doses may produce toxic side effects.

Table.1. Predicted biological activities of Benzofuran-2-one, 4-amino-2, 3-dihydro- at Pa> 70%

Pa	Pi	Activity
0.887	0.008	Methylenetetrahydrofolate reductase (NADPH) inhibitor
0.853	0.003	Cholestanetriol 26-monooxygenase inhibitor
0.809	0.001	Paraoxonase substrate
0.812	0.029	Ubiquinol-cytochrome-c reductase inhibitor
0.726	0.007	CYP2B5 substrate
0.737	0.021	NADPH peroxidase inhibitor
0.761	0.045	Aspulvinone dimethylallyltransferase inhibitor
0.730	0.014	Complement factor D inhibitor
0.757	0.045	Membrane integrity agonist
0.711	0.006	CYP2A11 substrate
0.747	0.048	CYP2C12 substrate
0.702	0.008	4-Nitrophenol 2-monooxygenase inhibitor
0.724	0.065	Phobic disorders treatment

Table.1.1. Predicted Toxic effects of Benzofuran-2-one, 4-amino-2, 3-dihydro- at pa.70%

Pa	Pi	Activity
0.758	0.018	Nail discoloration
0.726	0.009	Hematuria
0.714	0.039	Pure red cell aplasia
0.704	0.076	Shivering
0.707	0.096	Twitching

Table.2. Predicted biological activities of 8-Octadecenoic acid, methyl ester at Pa> 70%

Pa	Pi	Activity
0.948	0.001	All-trans-retinyl-palmitate hydrolase inhibitor
0.944	0.003	Chymosin inhibitor
0.933	0.002	GST A substrate
0.926	0.003	CYP2J substrate
0.922	0.004	Antieczematic
0.912	0.005	Polyporopepsin inhibitor
0.905	0.004	Acylcarnitine hydrolase inhibitor
0.895	0.005	Mucomembranous protector
0.882	0.009	Ubiquinol-cytochrome-c reductase inhibitor
0.879	0.009	Phobic disorders treatment
0.864	0.003	Phosphatidylglycerophosphatase inhibitor
0.862	0.002	Preneoplastic conditions treatment
0.860	0.009	Mucositis treatment
0.856	0.009	Sugar-phosphatase inhibitor
0.849	0.004	Lipid metabolism regulator
0.853	0.011	Alkenylglycerophosphocholine hydrolase inhibitor
0.844	0.003	Macrophage colony stimulating factor agonist
0.844	0.006	Alkylacetylglycerophosphatase inhibitor
0.824	0.011	Beta-adrenergic receptor kinase inhibitor
0.824	0.011	G-protein-coupled receptor kinase inhibitor
0.816	0.002	Alcohol O-acetyltransferase inhibitor
0.815	0.005	Phosphatidylcholine-retinol O-acyltransferase inhibitor
0.834	0.024	Aspulvinone dimethylallyltransferase inhibitor
0.805	0.004	Sarcosine oxidase inhibitor
0.789	0.005	Lipoprotein lipase inhibitor
0.783	0.003	GST M substrate
0.782	0.003	GABA aminotransferase inhibitor
0.780	0.003	Platelet aggregation stimulant
0.774	0.005	Gluconate 5-dehydrogenase inhibitor
0.769	0.003	Leukotriene-B4 20-monooxygenase inhibitor
0.773	0.010	Exoribonuclease II inhibitor
0.771	0.008	IgA-specific serine endopeptidase inhibitor
0.762	0.006	Fatty-acyl-CoA synthase inhibitor
0.757	0.003	Gastrin inhibitor
0.758	0.005	Antisecretoric
0.755	0.004	HMOX1 expression enhancer
0.767	0.017	Sphinganine kinase inhibitor
0.764	0.015	TP53 expression enhancer
0.754	0.006	Vasodilator. Peripheral
0.759	0.012	Prostaglandin-E2 9-reductase inhibitor
0.752	0.005	Cholesterol antagonist
0.753	0.007	Vasoprotector
0.746	0.001	Linoleate isomerase inhibitor
0.745	0.002	Vanilloid 1 agonist
0.748	0.006	Glucan 1.4-alpha-maltotriohydrolase inhibitor
0.749	0.007	Dextranase inhibitor

0.764	0.027	Chlordecone reductase inhibitor
0.753	0.020	Membrane permeability inhibitor
0.729	0.003	BRAF expression inhibitor
0.735	0.009	Acetylesterase inhibitor
0.728	0.005	Lactase inhibitor
0.725	0.004	Cytoprotectant
0.724	0.006	Phenol O-methyltransferase inhibitor
0.717	0.002	Antiinflammatory. Intestinal
0.737	0.024	Taurine dehydrogenase inhibitor
0.710	0.003	Plasmanylethanolamine desaturase inhibitor
0.716	0.028	Methylenetetrahydrofolate reductase (NADPH) inhibitor
0.729	0.046	Testosterone 17beta-dehydrogenase (NADP+) inhibitor

Table.2.1. Predicted Toxic effects of 8-Octadecenoic acid, methyl ester at Pa>70%

Pa	Pi	Activity
0.970	0.002	Shivering
0.922	0.003	Apnea
0.904	0.010	Conjunctivitis
0.848	0.008	Edema
0.847	0.016	Euphoria
0.821	0.008	Hyperthermic
0.816	0.005	Eye irritation
0.810	0.003	Lacrimal secretion stimulant
0.806	0.004	Skin irritative effect
0.814	0.023	Diarrhea
0.802	0.021	Ocular toxicity
0.760	0.010	Keratopathy
0.748	0.018	Muscle weakness
0.781	0.052	Twitching
0.746	0.024	Weakness
0.741	0.021	Anemia
0.728	0.030	Reproductive dysfunction
0.714	0.039	Ulcer

Table.3. Predicted biological activities of Stigmastanol at Pa> 70%

Pa	Pi	Activity
0.983	0.001	Alkylacetylglycerophosphatase inhibitor
0.981	0.001	Acylcarnitine hydrolase inhibitor
0.981	0.001	Alkenylglycerophosphocholine hydrolase inhibitor
0.961	0.002	Antihypercholesterolemic
0.951	0.001	Cholesterol antagonist
0.939	0.000	CYP7 inhibitor
0.939	0.001	Cholestanetriol 26-monooxygenase inhibitor
0.937	0.003	Protein-disulfide reductase (glutathione) inhibitor
0.932	0.001	Alkenylglycerophosphoethanolamine hydrolase inhibitor
0.933	0.003	Anesthetic general
0.926	0.001	CYP4B substrate
0.920	0.001	Peptidoglycan glycosyltransferase inhibitor
0.916	0.002	UGT2B1 substrate
0.916	0.004	Hypolipemic
0.912	0.001	Adenomatous polyposis treatment
0.901	0.006	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0.897	0.003	Lipoprotein lipase inhibitor
0.890	0.002	Sulfite dehydrogenase inhibitor
0.887	0.002	Choleretic
0.883	0.000	Cholesterol oxidase inhibitor
0.884	0.003	D-lactaldehyde dehydrogenase inhibitor

0.878	0.002	Hydroxylamine reductase (NADH) inhibitor
0.875	0.005	Respiratory analeptic
0.872	0.003	Glyceryl-ether monooxygenase inhibitor
0.866	0.001	Bile-salt sulfotransferase inhibitor
0.867	0.004	Caspase 3 stimulant
0.863	0.002	Vitamin-K-epoxide reductase (warfarin-insensitive) inhibitor
0.861	0.004	UDP-glucuronosyltransferase substrate
0.863	0.006	CYP2C substrate
0.859	0.002	UGT1A4 substrate
0.858	0.003	Hepatoprotectant
0.848	0.010	CYP3A substrate
0.839	0.002	Thiosulfate dehydrogenase inhibitor
0.825	0.002	Flavin-containing monooxygenase inhibitor
0.827	0.005	Oxidoreductase inhibitor
0.824	0.005	CYP3A inducer
0.815	0.001	Morphine 6-dehydrogenase inhibitor
0.822	0.014	Antieczematic
0.813	0.007	CYP2B6 substrate
0.806	0.004	CYP2C11 substrate
0.806	0.004	Chemopreventive
0.802	0.003	CYP4A11 substrate
0.798	0.003	Aldehyde dehydrogenase (NADP+) inhibitor
0.791	0.001	Retinol O-fatty-acyltransferase inhibitor
0.797	0.010	Membrane permeability inhibitor
0.786	0.002	Phospholipase C inhibitor
0.779	0.004	Antipruritic
0.777	0.003	Cytoprotectant
0.771	0.008	CYP3A5 substrate
0.762	0.001	Cholesterol synthesis inhibitor
0.761	0.001	Alkyl glycerol phosphor ethanolamine phosphor diesterase inhibitor
0.779	0.026	CYP2J substrate
0.756	0.007	Analeptic
0.746	0.003	Erythropoiesis stimulant
0.736	0.005	Bone diseases treatment
0.733	0.003	Thiamine-triphosphatase inhibitor
0.734	0.005	Antiosteoporotic
0.744	0.023	CYP2J2 substrate
0.719	0.004	Biliary tract disorders treatment
0.713	0.005	Proliferative diseases treatment
0.720	0.014	Immunosuppressant
0.702	0.001	Cholate-CoA ligase inhibitor
0.702	0.005	Antiviral (Influenza)

Table.3.1. Predicted Toxic effects of Stigmastanol at Pa>70%

Pa	**Pi**	**Activity**
0.928	0.008	Conjunctivitis
0.923	0.008	Sleep disturbance
0.921	0.006	Reproductive dysfunction
0.887	0.010	Drowsiness
0.842	0.009	Embryotoxic
0.838	0.015	Ocular toxicity
0.821	0.013	Excitability
0.818	0.015	Xerostomia
0.807	0.012	Asthma
0.807	0.022	Nausea
0.787	0.004	Cataract

0.803	0.021	Sensory disturbance
0.794	0.023	Emetic
0.789	0.026	Behavioral disturbance
0.778	0.026	Dermatitis
0.767	0.025	Headache
0.729	0.024	Consciousness alteration

Table.4. Predicted biological activities of dodecanoic acid 10-methyl- methyl ester at Pa>70%

Pa	Pi	Activity
0.951	0.002	Chymosin inhibitor
0.922	0.004	Polyporopepsin inhibitor
0.917	0.004	Phobic disorders treatment
0.884	0.005	Acylcarnitine hydrolase inhibitor
0.872	0.011	Ubiquinol-cytochrome-c reductase inhibitor
0.847	0.003	Preneoplastic conditions treatment
0.814	0.004	All-trans-retinyl-palmitate hydrolase inhibitor
0.804	0.005	Lipid metabolism regulator
0.807	0.013	5 Hydroxytryptamine release stimulant
0.809	0.023	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0.804	0.020	CYP2J substrate
0.789	0.011	GST A substrate
0.784	0.011	Alkylacetylglycerophosphatase inhibitor
0.763	0.004	GABA aminotransferase inhibitor
0.778	0.020	Sugar-phosphatase inhibitor
0.787	0.037	Aspulvinone dimethylallyltransferase inhibitor
0.740	0.004	Platelet aggregation stimulant
0.733	0.002	Sclerosant
0.746	0.022	Membrane permeability inhibitor
0.756	0.033	Mucomembranous protector
0.729	0.009	Acetylesterase inhibitor
0.735	0.020	Mucositis treatment
0.720	0.008	Vasodilator. peripheral
0.716	0.006	Anesthetic general
0.719	0.022	TP53 expression enhancer
0.719	0.039	Antieczematic

Table.4.1. Predicted Toxic effects of dodecanoic acid 10-methyl- methyl ester at Pa>70%

Pa	Pi	Activity
0.947	0.003	Shivering
0.891	0.003	Eye irritation, weak
0.876	0.004	Skin irritative effect
0.873	0.014	Conjunctivitis
0.831	0.011	Muscle weakness
0.777	0.011	Apnea
0.785	0.023	Euphoria
0.741	0.020	Acidosis, metabolic
0.740	0.028	Reproductive dysfunction
0.743	0.034	Pure red cell aplasia
0.731	0.032	Ocular toxicity
0.723	0.036	Sleep disturbance
0.702	0.036	Drowsiness

Table.5. Predicted biological activities of octadecanoic acid, 3-hydroxy-2-(1-oxotetradecyl), methyl ester at Pa>70%

Pa	Pi	Activity
0.955	0.002	Chymosin inhibitor
0.941	0.003	Polyporopepsin inhibitor
0.862	0.014	Ubiquinol-cytochrome-c reductase inhibitor
0.850	0.005	GST A substrate
0.833	0.010	Acylcarnitine hydrolase inhibitor
0.826	0.004	Acetylesterase inhibitor
0.813	0.008	Alkylacetylglycerophosphatase inhibitor
0.813	0.012	Sphinganine kinase inhibitor
0.796	0.005	Macrophage colony stimulating factor agonist
0.770	0.004	Preneoplastic conditions treatment
0.790	0.037	Phobic disorders treatment
0.760	0.023	Sugar-phosphatase inhibitor
0.739	0.004	Platelet aggregation stimulant
0.760	0.031	CYP2J substrate
0.719	0.005	Alcohol dehydrogenase (acceptor) inhibitor
0.716	0.004	Mycothiol-S-conjugate amidase inhibitor
0.717	0.008	Vasodilator, peripheral
0.707	0.053	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0.702	0.057	CYP2C12 substrate

Table.5.1. Predicted Toxic effects of octadecanoic acid, 3-hydroxy-2-(1-oxotetradecyl), methyl ester at Pa>70%

Pa	Pi	Activity
0.967	0.002	Skin irritation, weak
0.930	0.008	Conjunctivitis
0.881	0.010	Shivering
0.848	0.014	Ocular toxicity
0.804	0.008	Apnea
0.739	0.028	Reproductive dysfunction
0.711	0.005	Spermicide
0.703	0.020	Edema

4. CONCLUSION

Our study using PASS has been quite successful in revealing the anticancer treatment potential of the PHF and it also indicates a few side effects like reproductive dysfunction, skin irritation and ocular toxicity. Further *in vitro* and *in vivo* studies are to be done to validate these predictions.

5. ACKNOWLEDGEMENT

We take this opportunity to acknowledge and thank DST-INSPIRE (K.KAVYA – IF NO: 130433) for the financial support provided in the form of JRF and SRF and would also like to thank Lanson Biotech for helping us prepare the Polyherbal formulation.

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