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Prediction of biological activity spectra of a few phytoconstituents of *Azadirachta indicia* A. Juss

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Abstract

The use of drug substances derived from plants, fungi, bacteria, and marine organisms are ‘‘Mother Nature Gift’’ for diseases of mankind. Many of these are discovered serendipitously and have a long tradition in medicine. Till date, the use of natural products, their semi synthetic and synthetic derivatives have been mostly confined to their ethnic use. But it has been well known that each substance has a wide spectrum of biological activities as evident from some new uses of many old drugs. PASS (Prediction of Activity Spectra for Substances) has been employed as a strong potential tool to predict the biological activity spectrum of synthetic substances for the discovery of new drugs. But the potential of PASS to predict the biological activity spectra of natural products is still underestimated. The present study was therefore undertaken to investigate and correlate the biological activity spectrum of the main phytoconstituent of ***Azadirachta indica*** with their reported biological activities in order to evaluate the applicability of PASS.

Keywords: Biological activity spectrum, ***Azadirachta indica,*** PASS.

Introduction

Natural products (NPs) are used in folk medicine since many thousands year, due to their biological origin, better ADME/T (absorption, distribution, metabolism, and excretion/toxicity) characteristics and high chemical diversity. Presently, NPs are considered as a valuable source of lead structures for new pharmaceutical agents. Over 70% of New Chemical Entities (NCEs) introduced into medical practice from 1981–2006 were obtained on the basis of NPs (Newman and Cragg, 2007). NPs have always represented a significant, though often underappreciated resource for the development of new medicines. Even now, in combinatorial chemistry era, drugs from plants or microbial origin account for more than 30% of worldwide sale. Moreover, NPs have been notably successful in the past in opening up new avenues of exploration and in producing entirely new therapeutic classes (Grabley and Thiericke, 1999; Sneader, 2005).

Virtual screening is of particular significance to understand pharmacological behavior and receptor interactions of plant compounds (Rollinger *et al.,* 2008). A lot of empirical knowledge about pharmacotherapeutic properties of NPs is accumulated in Traditional Indian Medicine (TIM) ‘‘Ayurveda’’, which is known earlier than 1000 years BC. Currently, this empirical knowledge could be analyzed using up-to-date computational and experimental approaches. Such studies could give information about the basic mechanisms of TIM actions, providing the basis for rational design of new medicinal plant combinations, individualization of therapy taking into account particular geno- & phenotypes, and identification of new lead compounds for future pharmaceuticals. Today hundreds of known pharmacological targets are used in medicine and NPs exhibits a pleiotropic action by interacting with these multiple targets. All these facts emphasize that computer-aided methods could be extremely useful in pharmacological evaluation of NPs (Rollinger *et al.,* 2009). The global research scenario suggests the use of virtual screening techniques for the discovery of bioactive phytoconstituents. Currently in India, herbal drug research has been focused to standardize herbal drugs via the principles of ‘‘reverse pharmacology’’ leading to the integration of Ayurveda with modern medicine (Patwardhan *et al.,* 2004).

But all these strategies end up with the identification of phytochemical lead confined to its ethnic use only. In this regard, an effort has been made to explore more comprehensive pharmacological profile of phytoconstituents by application of a computer program PASS (Prediction of Activity Spectra for Substances). The proposed *in - silico* approach extends further to reveal novel biological activities of selected phytochemical leads, their mechanisms and related side-effects. PASS applicability to

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NPs has been demonstrated in some investigations (Dembitsky *et al.,* 2005; Zotchev *et al.,* 2006). The current version of PASS predicts around 3750 pharmacological effects, biochemical mechanisms of action, specific toxicities and metabolic terms on the basis of structural formulae of drug-like substances with average accuracy *95%. This can be further validated in in-vitro as well as in-vivo assays (Filimonov and Poroikov, 2008; Poroikov *et al.,* 2009).

This study involves the use of PASS for exploring the hidden pharmacological potential of selected traditional Indian medicinal plant *Azadirachta indica* based on their main phytoconstituents.

Neem is a native tree of India, found in every part in India especially in semi-arid conditions. The neem tree is an incredible plant that has been declared the "Tree of the 21st century" by the United Nations. In India, it is variously known as "Divine Tree", "Life giving tree", "Nature's Drugstore", "Village Pharmacy" and "Panacea for all diseases". It is one of the major components in Ayurvedic medicine, which has been practiced in India since many centuries. The neem is an ancient Indian cure-all due to its antibacterial, antifungal, antiviral, antihistamine and antiseptic properties. The neem leaves, flowers, seeds, roots, bark and fruits are utilized to treat inflammation, infections, skin diseases and for dental care.

Material and methods

Materials

Nimbin, Ninbidol, Gedunin, Salannin, Azadirachtin and azadirone from ***Azadirachta indica*** L were selected as main phytoconstituents based on literature reports. The structures of these phytoconstituents were obtained from National Centre for Biotechnology Information (NCBI) and reported literature. An extensive literature search was carried out to collect information about the common biological activities of these plants and their individual phytoconstituents using various databases (PubMed, ScienceDirect, DNP, etc.).

Methods

The chemical structures of the selected phytoconstituents of *Azadirachta indica* were obtained from NCBI (National Centre for Biotechnology Information; **www.ncbi.nlm.nih.gov/**). The selected structures were downloaded as sdf file for the prediction in PASS programme.

PASS (Prediction of Activity spectra for Substances)

The biological activity spectra of these phytoconstituents were obtained by PASS version (version 9.1, http://195.178.207.233/PASS). This software estimates the predicted activity spectrum of a compound as probable activity (Pa) and probable inactivity (Pi). Prediction of this spectrum by PASS is based on SAR analysis of the training set containing more than 205,000 compounds exhibiting more than 3750 kinds of biological activities. Being probabilities, the Pa and Pi values vary from 0.000 to 1.000 and, in general, Pa +Pi≠1, since these probabilities are calculated independently. The PASS prediction results were interpreted and used in a flexible manner: (i) only activities with Pa>Pi are considered as possible for a particular compound; (ii) if Pa>0.7, the chance to find the activity experimentally is high; (iii) if 0.5<Pa<0.7, the chance to find the activity experimentally is less, but the compound is probably not so similar to known pharmaceutical agents; (iv) if Pa<0.5, the chance to find the activity experimentally is less, but the chance to find a structurally new compound, that is, NCEs is more (Marwaha *et al.,* 2007).

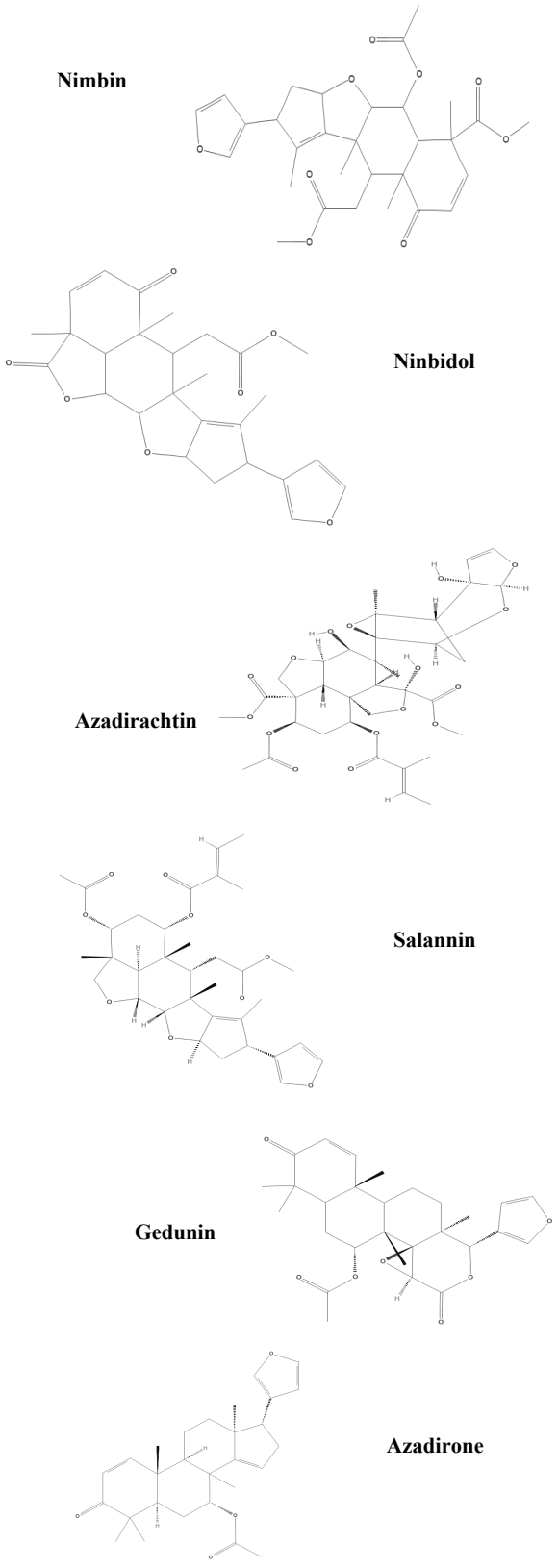

Figure 1: Structures of the selected phytoconstituents of *Azadirachta indica*

Results and discussion

For thousands of years the beneficial properties of Neem (*Azadirachta indica*) have been recognized in the Indian tradition. Each part of the neem tree has some medicinal property. Biswas *et al* (2002) have recently reviewed the biological activities some of the neem compounds, pharmacological actions of the neem extracts, clinical study and plausible medicinal applications of neem along with their safety evaluation.

Neem has been extensively used in ayurveda, unani and homoeopathic medicine. The Sanskrit name of neem tree is *Arishtha* meaning 'reliever of sickness' and hence is considered as *Sarbaroganibarini*. The tree is still regarded as 'village dispensary' in India. The importance of the neem tree has been recognized by US National Academy of Sciences, which published a report in 1992 entitled 'Neem – a tree for solving global problems'.

More than 135 compounds have been isolated from different parts of neem and several reviews have also been published on the chemistry and structural diversity of these compounds. The compounds have been divided into two major classes: isoprenoids (like diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and C- secomeliacins such as nimbin, salanin and azadirachtin) and non-isoprenoids, which are proteins (amino acids) and carbohydrates (polysaccharides), sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds, etc. In the present study, the chemical compounds of Nimbin, Ninbidol, Gedunin, Salannin, Azadirachtin and azadirone of neem tree were obtained from NCBI and predicted using PAAS programme.

PASS Results

The neem tree has been a chemist's paradise and it yields a variety of compounds, totaling about 300, belonging to the chemical classes of diterpenes, limonoids (tetranortriterpenoids), sulphur compounds, flavonoids, amino acids and carbohydrates.

Table 1: Nimbin

61 Substructure descriptors; 1 new.
29 Possible activities at Pa > 30%

Pa	Pi	Activity
0.823	0.016	Phosphatase inhibitor
0.675	0.008	Apoptosis agonist
0.652	0.046	Antineoplastic (lymphoma)
0.639	0.036	Antineoplastic (non-Hodgkin's lymphoma)
0.586	0.018	Cardiotonic
0.479	0.018	Beta glucuronidase inhibitor
0.502	0.068	Smooth muscle myosin light chain kinase inhibitor
0.437	0.026	Myc inhibitor
0.441	0.062	Renal failure treatment
0.424	0.053	Polarisation stimulant
0.365	0.046	Chemopreventive
0.342	0.027	Transactivator transcription protein inhibitor
0.353	0.048	Prostate cancer treatment
0.432	0.170	Antineoplastic (gastric cancer)
0.394	0.133	Hypokalemia

Table 2: Ninbidol

55 Substructure descriptors; 1 new.
23 Possible activities at Pa > 30%

Pa	Pi	Activity
0.827	0.015	Phosphatase inhibitor
0.750	0.003	Beta glucuronidase inhibitor
0.495	0.024	Apoptosis agonist
0.499	0.031	Polarisation stimulant
0.456	0.021	Myc inhibitor
0.394	0.005	Transcription factor inhibitor
0.381	0.032	CYP2A11 substrate
0.408	0.074	Antineoplastic
0.411	0.084	GST M substrate
0.436	0.116	Smooth muscle myosin light chain kinase inhibitor
0.346	0.061	Serine protease unspecified inhibitor
0.362	0.078	Interleukin 5 antagonist
0.313	0.032	CF transmembrane conductance regulator agonist
0.347	0.074	T cell inhibitor
0.346	0.078	Cardiotonic

Neem elaborates a vast variety of biologically active compounds which are chemically diverse and structurally complex.

Traditionally the following phytoconstituents Nimbin used for anti-inflammatory, anti-pyretic, anti-histamine, anti-fungal, Ninbidol for anti-tubercular, anti-protozoan, anti-pyretic, Gedunin for vasodilator, anti-malarial, anti-fungal, Salannin for insect repellent and Azadirachtin for insect repellent, anti-feedant, anti-hormonal. Azadirone is isolated from neem oil and possess only low antifeedant activity.

Table 3: Gedunin

54 Substructure descriptors; 0 new.
55 Possible activities at Pa > 30%

Pa	Pi	Activity
0.914	0.004	Cytochrome P450 inhibitor
0.857	0.010	Phosphatase inhibitor
0.845	0.004	CYP3A4 inhibitor
0.708	0.006	Chemopreventive
0.594	0.006	Beta glucuronidase inhibitor
0.604	0.017	TERT expression inhibitor
0.554	0.016	Apoptosis agonist
0.507	0.011	Myc inhibitor
0.503	0.009	Nitric oxide antagonist
0.536	0.051	Smooth muscle myosin light chain kinase inhibitor
0.467	0.004	Transactivator transcription protein inhibitor
0.492	0.042	Menstruation disorders treatment
0.484	0.035	Menopausal disorders treatment
0.471	0.025	Caspase 8 stimulant
0.441	0.015	Gestagen antagonist

The results of this study justice the applicability of PASS program for the prediction of biological activities of a plant, based on its main phytoconstituent, as evidenced by an average prediction coefficient of 0.85 for the selected six phytoconstituents. Further, it was found that some of the reported activities of the plant were not predicted by the PASS. Phosphatase inhibitor, Apoptosis agonist,

anitneoplastic activities were found in all the phytoconstituents of neem tree in the prediction. These activities coefficients differed from one compound to another. Myc inhibitor, chemo preventive and cardio tonic activities were also found in four compounds. The phytoconstituents obtained from the same plant neem, so their activity also showed some resemblance. The first 15 biological activity spectrum of the six phytoconstituents was represented in the tables (Table 1-6).

Table 4: Salannin

64 Substructure descriptors; 0 new.
23 Possible activities at Pa > 30%

Pa	Pi	Activity
0.820	0.016	Phosphatase inhibitor
0.753	0.003	Beta glucuronidase inhibitor
0.475	0.017	Myc inhibitor
0.476	0.028	Apoptosis agonist
0.485	0.052	Protein-arginine deiminase inhibitor
0.483	0.052	Antineoplastic
0.407	0.005	Transcription factor inhibitor
0.425	0.034	Mitochondrial electron transport inhibitor
0.394	0.037	Hepatic disorders treatment
0.427	0.071	GST M substrate
0.385	0.034	TERT expression inhibitor
0.408	0.058	Bilirubin oxidase inhibitor
0.347	0.024	Transactivator transcription protein inhibitor
0.363	0.056	Hypercalcaemic
0.332	0.028	Nitric oxide antagonist

Though plant phytoconstituent exhibit enormous structural diversity, only a small proportion of that diversity has been seriously explored for its pharmacological potential so far and there is, therefore, a little reason to believe that this potential has now run dry. As a phytochemical entity is capable of depicting a lot of biological activities but only a bit of it had been explored based upon its traditional use or serendipitously (Grabley and Thiericke, 1999; Elvin-Lewis, 2001). Moreover, it is also not feasible to explore all the biological activities based on hit and trial basis. PASS can be a possible approach to determine the novel possible activities of the existing phytoconstituents. In the study on examination of the PASS spectrum of the selected phytoconstituents of *Azadirachta indica*, it was found that there were a significant number of unexplored pharmacological activities in each constituent. As the PASS-predicted pharmacological activities with a score of Pa>0.7 have very high chances to be obtained experimentally, therefore only the unexplored pharmacological activities with a score of Pa>0.7 have been summarized and listed as hidden pharmacological potential of these compounds.

Table 5: Azadirachtin

78 Substructure descriptors; 7 new.
48 Possible activities at Pa > 30%

Pa	Pi	Activity
0.853	0.005	Antineoplastic
0.788	0.004	CYP2A11 substrate
0.748	0.033	Phosphatase inhibitor
0.673	0.009	Cytostatic
0.651	0.017	Immunosuppressant
0.621	0.014	Antifungal
0.616	0.009	Polarisation stimulant
0.611	0.035	Antiinflammatory
0.534	0.008	Myc inhibitor
0.559	0.034	CYP2C19 inducer
0.587	0.082	Antineoplastic (non-small cell lung cancer)
0.492	0.004	Transcription factor inhibitor
0.505	0.022	Apoptosis agonist
0.515	0.040	Protein-arginine deiminase inhibitor
0.499	0.029	Menopausal disorders treatment

Table 6: Azadirone

49 Substructure descriptors; 0 new.
113 Possible activities at Pa > 30%

Pa	Pi	Activity
0.859	0.003	Chemopreventive
0.846	0.003	Nitric oxide antagonist
0.835	0.014	Phosphatase inhibitor
0.808	0.009	Antiinflammatory
0.735	0.005	Apoptosis agonist
0.685	0.043	Reproductive dysfunction
0.668	0.030	Oxidoreductase inhibitor
0.646	0.014	TERT expression inhibitor
0.629	0.020	Immunosuppressant
0.657	0.057	Mucomembranous protector
0.617	0.021	Smooth muscle myosin light chain kinase inhibitor
0.646	0.051	Hypokalemia
0.597	0.010	Antiviral (Influenza)
0.589	0.011	Hepatoprotectant
0.578	0.013	CYP17 inhibitor

Conclusion

Using the computer programme PASS, the biological spectrum activity of the selected phytoconstituents Nimbin, Ninbidol, Gedunin, Salannin, Azadirachtin and azadirone from ***Azadirachta indica*** were analyzed. PASS use in drug R&D might provide selecting compounds which structure is not similar to well known drugs and estimating probable activity spectra for new compound to optimize their synthesis and testing.

From the results of this study, it is concluded that PASS predictions well correspond to the reported activities of the main and some other phytoconstituents of selected medicinal plant. Previously unexplored but PASS-predicted activities provide the basis for evaluation of hidden potential of this medicinal plant.

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