Med Chem Res
DOI 10.1007/s00044-010-9398-y

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

PASS-assisted exploration of new therapeutic potential of natural products

Rajesh Kumar Goel · Damanpreet Singh · Alexey Lagunin · Vladimir Poroikov

Received: 15 March 2010 / Accepted: 22 July 2010

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 semisynthetic 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 some selected Indian medicinal plants with their reported biological activities in order to evaluate the applicability of PASS. Further, the unexplored but PASS-predicted activities having good activity score (Pa > 0.7) for particular structure were listed as hidden potential of the plant.

Keywords Ayurveda · Biological activity spectrum · Herbal drugs · Natural products · 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

Electronic supplementary material The online version of this article (doi:10.1007/s00044-010-9398-y) contains supplementary material, which is available to authorized users.

R. K. Goel (✉) · D. Singh
Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala 147002, Punjab, India
e-mail: goelrkpup@gmail.com; goelrk_us@ymail.com

A. Lagunin · V. Poroikov
Institute of Biomedical Chemistry of Russian Academy of Medical Sciences, 10, Pogodinskaya Street, Moscow 119121, Russia

Published online: 06 August 2010

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 emphasizes 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 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 plants based on their main phytoconstituents.

Materials and methods

Withania somnifera, *Curcuma longa*, *Boerhaavia diffusa*, *Piper longum*, and *Alium sativum* were selected for the study. Withanolide A from *Withania somnifera* (Malik *et al.*, 2007), Curcumin from *Curcuma longa* (Jurenka, 2009), Liriodendrin from *Boerhaavia diffusa*, Piperine from *Piper longum* (Bhardwaj *et al.*, 2002), and Allicin from *Allium sativum* (Zhang *et al.*, 2008) (Table 1) were selected as main phytoconstituents based on literature reports. The structures of these phytoconstituents were obtained from Dictionary of Natural Products (DNP) 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 (Supplement Table 1) using various databases (PubMed, ScienceDirect, DNP, etc.).

The biological activity spectra of these phytoconstituents were obtained by PASS version (version 9.1, http://www.ibmc.msk.ru/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, $Pa + Pi \neq 1$, since these probabilities are calculated independently.

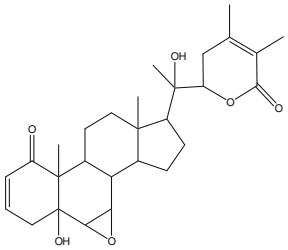
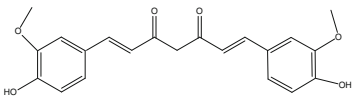
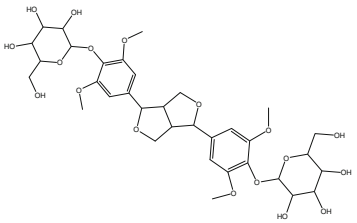
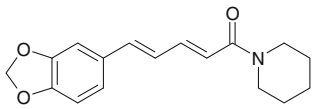
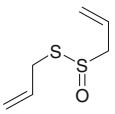
The PASS prediction results were interpreted and used in a flexible manner: (i) only activities with $Pa > Pi$ are considered as possible for a particular compound; (ii) if $Pa > 0.7$, the chance to find the activity experimentally is high; (iii) if $0.5 < Pa < 0.7$, the chance to find the activity experimentally 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). In this study, PASS prediction results (P_2) were analyzed and compared with the reported activities of plant (P_1), to obtain prediction coefficient (P) for each plant's main phytoconstituent. Then the unpredicted but already reported activities were matched with the PASS-predicted biological spectrum (P_3) of other known phytoconstituents in order to obtain the corrected prediction coefficient (P*) of the particular plant for further consideration of PASS applicability (Table 2; Supplement Table 1). Finally the unexplored but PASS predicted activities having score $Pa > 0.7$ for particular structure were listed as a hidden potential of the plant (Table 3).

Results and discussion

Withania somnifera

Withania somnifera is commonly known as Ashwagandha or Winter Cherry (Andallu and Radhika, 2000). It is a green shrub (family Solanaceae) found throughout the dry parts of India, Baluchistan, Pakistan, Afghanistan, Sri Lanka, Congo, South Africa, Egypt, Morocco, and Jordan (Dafni and Yaniv, 1994). The practitioners of the traditional system of medicine in India regard *Withania somnifera* as the "Indian Ginseng" (Singh *et al.*, 2001). Various parts of the plant have been used for centuries to alleviate variety of ailments (Bhattacharya *et al.*, 1987, 2001; Kulkarni *et al.*, 1998). Many pharmacological studies have been carried out in past to describe its multiple biological properties, many of which are indexed in the

Table 1 Names of plants and their main bioactive phytoconstituent selected for PASS prediction in this study

S. no	Plant name	Common name	Selected main phytoconstituent	Structure of the selected main phytoconstituent
1	*Withania somnifera*	Ashwgandha	Withanolide A	
2	*Curcuma longa*	Turmeric	Curcumin	
3	*Boerhaavia diffusa*	Punarnava	Liriodendrin	
4	*Piper longum*	Pipali	Piperine	
5	*Alium sativum*	Garlic	Allicin	

dictionary of natural products (Supplement Table 1). The major phytoconstituents of the plant are steroidal alkaloids and lactones, a class of constituents together known as withanolides (steroidal lactones with ergostane skeleton) (Elsakka *et al.*, 1990). Various alkaloids present in the plant include withanine, somniferine, somnine, somniferinine, withananine, psuedo-withanine, tropine, psuedotropine, 3-α-gloyloxytropane, choline, cuscohygrine, isopelletierine, anaferine, and anahydrine (Kulkarni *et al.*, 2008). Since withanolide A is the principal alkaloid present in the plant, therefore was selected to predict the biological spectrum of this plant by PASS. It was found that out of total 18 reported activities of the plant 13 were predicted by PASS for withanolide A (prediction coefficient 0.72) (Table 2). The remaining reported activities of *Wathania somnifera* which were not predicted by the PASS for withanolide A were correlated with the PASS predicted spectrum of the other reported phytoconstituents of this plant. It was found that out of remaining 05 activities all were predicted by PASS for the other phytoconstituents present in the plant further correcting the prediction coefficient to 1.00 (Table 2, Supplement Table 2).

Table 2 PASS prediction coefficient based on the main bioactive phytoconstituent from five selected medicinally active herbs

S. no	Plant name	Compound	P_1	P_2	P	P_3	P*
1	*Withania somnifera*	Withanolide A	18	13	0.72	05	1.00
2	*Curcuma longa*	Curcumin	29	21	0.72	02	0.79
3	*Boerhaavia diffusa*	Liriodendrin	22	10	0.45	07	0.77
4	*Piper longum*	Piperine	26	20	0.76	01	0.80
5	*Allium sativum*	Allicin	22	15	0.68	03	0.81

Compound, plant's selected main phytoconstituents; P_1, number of reported activities for the plant; P_2, number of PASS predicted activities for the compound coincided with the reported activities; $P = P_2/P_1$, prediction coefficient; P_3, number of PASS predicted activities for the other phytoconstituents coincided with the reported activities; $P^* = (P_2 + P_3)/P_1$, corrected prediction coefficient

Curcuma longa

Curcuma longa Linn., commonly known as Haldi, Turmeric, or Indian saffron, belongs to family Zingiberaceae. It is cultivated mostly in Ceylon, Belgium, Indonesia, France, and in many parts of India, especially in Bengal, Tamil Nadu, and Andhra Pradesh. *Curcuma longa* is a perennial herb with simple and large leaves. Its rhizomes and oil have great medicinal importance which has been summarized in Supplement Table 1. *Curcuma longa* contains a yellow compound curcumin, an essential oil (5%), an alkaloid, starch grains, curcuminoids, turmeric oil, turmerol, a coporioc acid, and veleric acid. The oil contains α- and β-curcumins, D-sallinene, α-phellandrene, cineol, zingiberene, small amount of sequiterpenes, α-and β-pinene, camphor and camphene (Jain *et al.*, 2007). In this study, PASS predicted 21 activities for curcumin out of total 29 reported activities of the plant (prediction coefficient 0.71) (Table 3). While considering the PASS spectrum of other constituents of *Curcuma longa*, we found that out of remaining 08 activities 02 were depicted by its other constituents, therefore further correcting its prediction coefficient to 0.79 (Table 2, Supplement Table 2).

Boerhaavia diffusa

Boerhaavia diffusa L. (Nyctaginaceae), commonly known as "Punarnava" in the Indian system of medicine, is a perennial creeping herb found throughout the waste land of India (Rawat *et al.*, 1997). It is used for its medicinal activities from antiquity, many of which have been experimentally validated (Supplement Table 1). Recently, we have evaluated its anticonvulsant activity in experimental animal models based upon its ethnomedical use (Kaur and Goel, 2009). *Boerhaavia diffusa* contains a large number of phytoconstituents such as, flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates,

Table 3 PASS predicted but not reported activities of selected phytoconstituents (hidden potential of medicinal plants)

S. no.	Selected phytoconstituent	Unexplored activities predicted by PASS (Pa, Pi)
1	Withanolide A	Cystic fibrosis treatment (0.904, 0.004)
		Antieczematic atopic (0.904, 0.004)
		Immunosuppressant (0.904, 0.004)
		Multiple sclerosis treatment (0.904, 0.004)
		Antipsoriatic (0.904, 0.004)
		Rheumatoid arthritis treatment (0.904, 0.004)
		Antieczematic (0.744, 0.028)
		Analgesic (0.904, 0.004)
		Antiallergic (0.904, 0.004)
		Antiacne (0.904, 0.004)
		Ulcerative colitis treatment (0.904, 0.004)
		Muscular dystrophy treatment (0.904, 0.004)
		Osteoarthritis treatment (0.904, 0.004)
		Neuroprotector (0.904, 0.004)
		Antipruritic (0.904, 0.004)
		Autoimmune disorders treatment (0.904, 0.004)
		Inflammatory bowel disease treatment (0.904, 0.004)
2	Curcumin	Cystic fibrosis treatment (0.930, 0.003)
		Antipsoriatic (0.930, 0.003)
		Allergic rhinitis treatment (0.718, 0.009)
		Antiviral (HIV and hepatitis B) (0.766 ,0.019)
		Antiasthmatic (0.930, 0.003)
		Antipruritic (0.766, 0.003)
		Inflammatory bowel disease treatment (0.930, 0.003)
		Radioprotector (0.766, 0.003)
		Anesthetic (0.766, 0.037)
		Mucositis treatment (0.902, 0.004)
3	Liriodendrin	Smooth muscle relaxant (0.814, 0.003)
		Reproductive dysfunction (0.811, 0.021)
		Vasoprotector (0.712, 0.018)
		Emetic (0.776, 0.044)
4	Piperine	Cystic fibrosis treatment (0.936, 0.002)
		Antipsoriatic (0.936, 0.002)
		Antiviral (HIV and Hepatitis B) (0.908, 0.009)
		Antiparkinsonian (0.813, 0.004)
		Antipruritic (0.908, 0.008)
		Anesthetic (0.908, 0.009)
		Alzheimer's disease treatment (0.936, 0.004)
		Antiepileptic (0.908, 0.004)
		Antiosteoporotic (0.718, 0.012)
		Prostate cancer treatment (0.740, 0.009)

Table 3 continued

S. no.	Selected phytoconstituent	Unexplored activities predicted by PASS (Pa, Pi)
5	Allicin	Hemostatic (0.744, 0.020)
		Antiulcerative (0.744, 0.013)
		Antiviral (0.751, 0.003)
		Antineoplastic (0.865, 0.001)
		Antileukemic (0.865, 0.001)
		Inflammatory Bowel disease treatment (0.851, 0.003)
		Septic shock treatment (0.744, 0.026)
		Antipruritic (0.831, 0.013)
		Antiprotozoal (0.744, 0.003)
		Atherosclerosis treatment (0.944, 0.003)
		Mucositis treatment (0.778, 0.024)
		Alopecia treatment (0.733, 0.007)
		Antiseborrheic (0.733, 0.046)

proteins, and glycoproteins (Sahu *et al.*, 2008). In the present study liriodendrin was selected as reference for the plant in PASS assessment. It was found that out of total 22 reported activities of the plant 10 were predicted by PASS for liriodendrin (prediction coefficient 0.45) (Table 2). The remaining activities which were not predicted by the PASS for liriodendrin but were reported for the plant were correlated with the PASS spectrum of other constituents present in the plant. It was found that out of remaining 12 activities 07 were predicted by PASS for the other phytoconstituents present in the plant raising the prediction coefficient to 0.77 (Table 2, Supplement Table 2).

Piper longum

Piper species are distributed widely in the tropical and subtropical regions of the world and have multiple therapeutic utilities in different folk medicine. *Piper longum* Linn. is an important member of genus Piper, which is widely used in traditional medicine by the population of Asia and Pacific islands. *Piper longum* is implicated in several disorders like, gonorrhea, menstrual pain, tuberculosis, sleeping problems, etc. (Supplement Table 1) (Sunila and Kuttan, 2004; Park *et al.*, 2007). Piperine is an amide derivative which was first isolated from piper species (Sunila and Kuttan, 2004) and is one of the major phytoconstituent of *Piper longum* (Bhardwaj *et al.*, 2002), therefore was selected for the present study. In this study, PASS predicted 20 activities for piperine in comparison to 26 reported activities of the plant, giving a prediction coefficient of 0.76 (Table 2). Further prediction coefficient was raised to 0.80 by correlating the remaining reported activities with the PASS spectrum of other phytoconstituents of *Piper longum* (Table 2, Supplement Table 2).

Allium sativum

Allium sativum Linn. (family Alliaceae), commonly known as garlic, is one of the best-researched, best-selling herbal remedies and is commonly used as a food and a spice (Pittler and Ernst, 2007). Active phytoconstituents present in *Allium sativum* include enzymes (e.g., alliinase), sulfur-containing compounds (e.g., alliin), and compounds synthesized enzymatically from alliin (e.g., allicin). Traditionally, it has been employed to treat infections, wounds, diarrhea, rheumatism, heart diseases, diabetes, and many other, majority of them have been experimentally validated (Supplement Table 1). As allicin is the major phytoconstituent of the plant (Zhang *et al.*, 2008), it was selected for the study. It was found that out of total 22 reported activities of *Allium sativum*, 15 were predicted by PASS for allicin (prediction coefficient 0.68) (Table 2). The remaining reported activities of *Allium sativum* that were not predicted by the PASS for allicin were correlated with the PASS-predicted activities for the other reported phytoconstituents of *Allium sativum*. It was found that out of remaining total 07 activities, 03 were predicted by PASS for the other phytoconstituents present in the *Allium sativum* (Supplement Table 2).

The results of this study justified 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 0.66 for the selected five plant's phytoconstituent. Further, it was found that some of the reported activities of the plant were not predicted by the PASS. The probable reason for this may be that reports available in the literature consisted whole plant or extract and the PASS predictions were based on the structure of the main phytoconstituent only. As the plant or its extract contains a number of phytoconstituents that themselves shows varied biological activities, therefore the reported activities can be due to the other phytoconstituents present in the plant or its extract. In this regard, some of the reported but not predicted activities of the selected plants was justified by this approach by comparing these reported activities with the PASS spectrum of other phytoconstituents. This approach improved prediction coefficient for each plant raising the average corrected prediction coefficient to 0.83 (Table 2, Supplement Table 2).

Though plant phytoconstituents 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 phytoconstituent of the selected plants, it was found that there were a significant number of unexplored pharmacological activities in each case. 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 plant (Table 3).

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 five selected medicinal plants. Previously unexplored but PASS-predicted activities provide the basis for evaluation of hidden potential of these medicinal plants.

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