Useful Methods for Targeted Plant Selection in the Discovery of Potential New Drug Candidates

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Abstract:

The efficient and effective selection of appropriate plants for investigative purposes in a drug discovery program is of crucial importance for a successful outcome. A variety of approaches have been used by researches with varying levels of success. A variety of different approaches to plant selection are discussed, including the ethnomedicinal approach, some ecological approaches and the use of combinatorial and computational methodologies.

Key words:

Biodiversity, targeted selection, ethnomedicine, drug discovery

Introduction

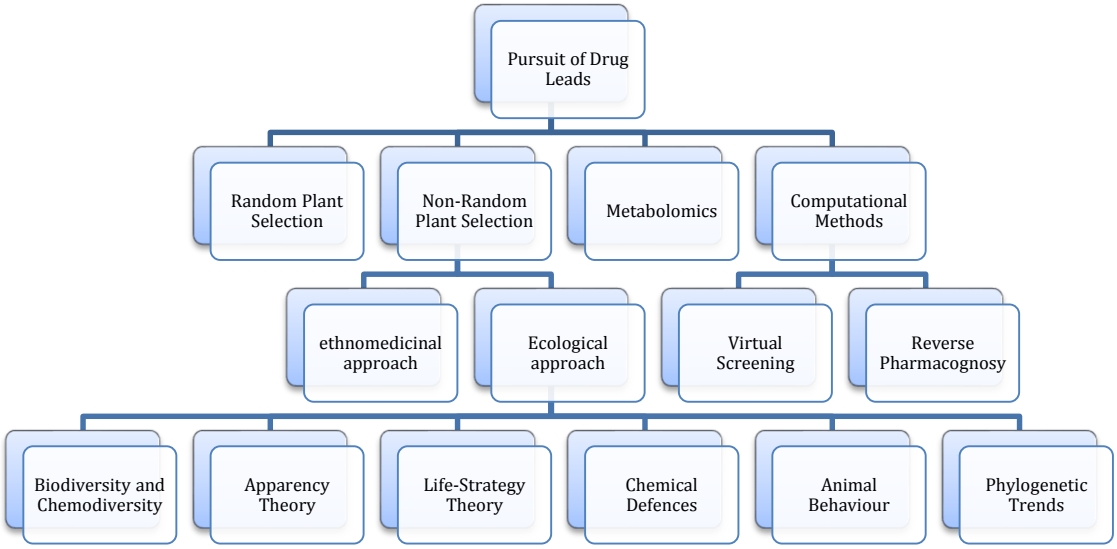

Figure 1: Outline of Possible Approaches to the Discovery of New Drug Leads

A review of the types of drugs used to treat the wide spectrum of both infectious and non-infectious diseases serves to highlight the essential role played by plant, marine and microorganism-based secondary metabolites. Undoubtedly natural sources still represent a rich untapped resource for the discovery of new drugs. The present lack of funding for natural products research in some parts of the world where this type of research is seen as 'fishing' and low citation numbers and relatively low impact factors of journals in the field make the area of research unpopular by some funding agencies and university administrators. On the other hand, in parts of the world where numbers of publications, and not necessary quality, is used as an indicator of academic success, often the rush to publish (and to publish as many papers as possible from one plant), and lack of appropriate screening facilities hinders the thorough evaluation of compounds produced for potential pharmacological activity. Faced with such extensive biodiversity and limited resources, the choice of where to most effectively focus one's attention becomes crucial. The choice may be guided by past successes or perhaps by current innovations with potential to deliver future results.

The success of natural product research has been well reviewed by many, including four thorough analyses of the contribution of natural products to approved therapeutic agents by Newman, Cragg and Snader in 1997 and 2003 [1, 2] and by Newman and

Cragg in 2007 and 2012 [3, 4]. During the period 1981 to 2010, 54% of all new approved drugs came from natural sources. Of these 4% were natural products, 22% were derived from natural products, 13% were produced by total synthesis but the active chromophore was a natural product and 15 % were from a biological source such as a large protein or peptide isolated from an organism or cell line [4]. If only the small-molecule approved drugs are considered, then 6% were natural products, 28% were derived from natural products and 16% were produced by total synthesis, where the pharmacophore was based on a natural product [4].

The development of drugs from natural products comes at a significant time and financial cost. It is estimated that on average the cost of getting a drug to market is in excess of $ 1 billion if post approval Phase IV costs and costs linked to approval on non US markets are taken into account [5]. The National Cancer Institute (NCI) screened around 200 000 extracts between 1955 and 1980 with limited success [6]. This led to a reduction in focus on random screening until 1986, when, with the improvement of screening methods, the NCI started screening again. By 1995, 40 000 extracts had been prepared and 18 000 screened for activity. The success rate was about 1% [6]. In contrast, however, combinatorial chemistry has resulted in only one approved drug (Sorafenib) in the public domain in the time period until 2010 [4]. This does not, however, take away from the fact that combinatorial chemistry is highly effective as a tool for structural optimization of an active chemical skeleton [4]. Comparing compounds prepared by people to those produced by plants, the most striking difference is one of complexity. Natural sources use enzymes to bring about chemical transformations, enabling very specific structural changes to be made to specific sites in a stereospecific manner resulting in a complex molecule [6]. Being able to tap into this source of chemical diversity in a meaningful and effective way is crucial to successful drug development.

The factors influencing the selection of marine or microbial samples for evaluation are, in most cases, different from those directing the choice of plant material. The marine environment provides a vast, largely untapped, source of biodiversity. One of the primary challenges facing sample selection in a marine setting is one of accessibility – your choice is often decided by what you can physically access and, as such, work is more frequently carried out on organisms living close to the shore [7].

Sampling techniques often require specialized equipment and in many cases very small quantities of active compound are obtained requiring nanomolar structure determination [7].

With approximately half of the 20 best–selling non-protein drugs based on natural products and with almost all of the currently used natural products being of terrestrial origin (Harvey 2000, in Monaster and Luesch 2011) [7], the identification of good methods to improve the successful selection of promising plant material remains crucial. In the light of the significant differences in the challenges facing the choice of marine or microbial material as opposed to plant material for study, this paper will restrict its comments to the selection of plant material.

Success in identifying a new biologically active plant-based natural product can be influenced firstly by a clever choice of plant to investigate or secondly by how quickly and effectively a random selection of plant extracts can be screened. Each of these approaches has produced a level of success and many of the methods employed in plant selection are based on one or the other system. The huge cost involved in both time and resources to screen vast numbers of randomly selected extracts, with the small success rate, has led many investigators to advocate selection based on various non-random approaches [8]. The various approaches that will be discussed are outlined in figure 1.

The ethnomedicinal approach

The ethnomedicinal approach has resulted in a number of success stories. The 19th century saw scientists starting to isolate the active principle from medicinally used plants – the first notable success being quinine from *Chinchona* bark by Caventou and Pelletier [9]. Other pre-world war 2 successes include morphine and codeine from the opium poppy, digoxin from *Digitalis* leaves and atropine (produced from (-)-hyoscyamine) from Solanaceae species [9]. Tiotropium is currently being used to treat chronic obstructive pulmonary disease and is a derivative of atropine [10]. More recent years have seen cancer therapy impacted by natural products. The most notable perhaps, being the derivatives of camptothecan and the diterpene, taxol. Camptothecan was isolated from *Campotheca acuminate* Decne., a tree widely used

in Chinese traditional medicine, which resulted in its inclusion in the National Cancer Institute screening program [10]. In contrast, several samples of *Taxus brevifolia* were randomly collected for analysis by the National Cancer Institute. The discovery of taxol was seen as serendipitous, but the tree has been used by West-American Indian groups for stomach complaints among other things and the Tsimshian (from British Columbia) use it to treat cancer [10].

Galanthamine, isolated from the Russian species *Galanthus woronowii* Losinsk. Was discovered through an ethnobotanical lead and is currently being used to treat Alzheimer's disease [10].

The decision to investigate a particular plant species is very often determined by the fact that the plant is already being used for some purpose, possibly medicinally or as an insect repellent or for some cultural purpose. This would constitute an ethnobotanical approach to plant selection and would apply to a vast number of phytochemical investigations. The testing of the plant extracts and any isolated compounds can be guided by what the plant is traditionally used for and any positive results would serve to validate the use of the plant as well as provide useful leads for further drug development.

The ethnomedicinal approach allows for an increased possibility of finding an active compound as well as a means of documenting and preserving local knowledge. This becomes of greater importance with the increased mobility among rural communities and the subsequent loss of local knowledge of the use of indigenous plant species [11]. Two important issues need to be addressed in regard to the ethnomedicinal approach to plant selection. Firstly the rights of the country of origin with respect to any drugs discovered need to be protected, as outlined in the United Nations Convention on Biological Diversity [12]. Secondly the quality of any ethnopharmacological field studies carried out prior to plant selection is important, and may have an impact on the success of the research [13]. Thorough ethnopharmacological field studies can lay a vital platform from which the phytochemical investigation of a plant species can be launched.

The development of ethnobotanical databases can provide valuable information to aid in plant selection for investigation. An example of this is a regional database detailing the 1672 uses (medicinal, ceremonial, veterinary) of 474 plant species in the Campania region of Italy compiled by De Natale *et al*. [14]. The researchers gathered the information from various historical sources, including diaries, travel accounts and treatises on medicinal plants over the last three centuries and noted that 50 plant species were continuously used over this time to treat the same ailments. A similar historical study was carried out by Giogetti *et al*. [15] on Brazilian plants used in relation to the central nervous system. A survey of historic books in various San Paulo libraries revealed thirty-four plant species, thirteen of which are also used by modern Brazilian communities. Only eight species have been studied from a pharmacological perspective. Perry *et al*. have similarly looked at historically used plants, mainly in Europe, to treat memory loss and Alzheimer's disease [16]. In addition to historical sources, databases of plant uses have been established by recording self-reported practices of local people. An example of this is the database started by Karunamoorthi *et al*. documenting the use of plants as insect-repellents in the Western Hararghe region of Ethiopia [17]. Lehman *et al.* [18] developed a systematic matrix that they used to compile a database of potential leads for pest management in Mali. Their criteria included traditional medicinal use; antimicrobial activity and insect defense activity and their information was gathered from the literature and interviews with local farmers, healers and scientists. Such databases can serve as a useful ethnobotanical starting point to plant selection for further testing and investigation. Hutchings *et al.'s* book on Zulu traditional plants [19], for example, has provided the basis of much work into plants used by the Zulu people.

Comprehensive field studies and the establishment of ethnomedicinal databases can provide valuable resources to those wishing to select promising plants for study. Statistical analysis of the sometimes vast databases of medicinally used plants has been successfully used to identify families of plants that are over- or underused by traditional practitioners. Regressional analysis [20], contingency table and binomial analyses [21] as well as Baysian and Imprecise Dirichlet Model (IDM) approaches [22, 23] have been used. Regression analysis has been used to analyse the SANBI Medlist Database of southern African medicinally used plants [20], contingency table and binomial analyses to investigate the Ecuadorian Shuar medicinal flora [21] and

the medicinal flora of Campania, Italy has been analysed by the Baysian, binomial and IDM methods [22, 23]. These studies all demonstrated a clear bias for particular families in each of the regions investigated and, as such, could be used to guide the choice of plants to be studied.

The value of the ethnomedicinal approach to plant selection may be determined by its successes and failures. A small number of studies will be mentioned here in this regard. Khafagi and Dewedar in 2000 and Gyllenhaal *et al*. in 2012 each directly compared the activity of plants collected randomly in a particular region with those selected in an ethnomedicinally-directed manner [24, 25]. Khafagi and Dewedar screened sixty plants growing wild in Sinai, Egypt for antibacterial and antifungal activity. Thirty-six were selected randomly and twenty-four were selected as the Bedouins in the region use them for their antibacterial or antifungal properties. Fifteen of the thirty-six randomly selected plants showed activity against some of the bacterial and fungal strains tested while twenty of the twenty-four ethomedicinally-selected plants showed activity (41.7% versus 83.3%) [24]. Gyllenhaal *et al*. compared plants randomly selected from the Cuc Phuong National Park in Vietnam with a selection of plants used by traditional healers of Laos and Vietnam. Two types of samples were investigated: 'samples', a single part of a plant species collected in a particular region and 'collections' which could contain more than one part of the plant, collected in a specific region. All extracts were screened for antimycobacterial, antiplasmoidal, chemopreventative and anticancer activity. The results were not overwhelmingly in favour of an ethnomedicinal approach to plant selection. Over the whole range of tests, the randomly selected 'collections' were 3% more likely to give a positive result than those ethnomedicinally selected. With 'samples', the ethnomedicinally selected extracts were 6% more likely to give a positive result [25].

A large number of papers have been published reporting positive activity over a wide range of tests for plant extracts chosen using ethnomedicinal criteria. In many cases the active principal is isolated and identified, but the value is mostly in verifying the efficacy of the plant rather than the isolated active compound being developed further into a registered drug. The ethnomedicinal approach has successfully been used by the researchers at Shaman Pharmaceuticals to verify the use of *Cryptolepsis*

sanguinolenta (Lindl.) as a treatment for type II diabetes as well as a basis for the isolation of the active component, the alkaloid, cryptolepine [26, 27]. *C. sanguinolenta* is used by traditional healers in Ghana to treat symptoms of type II diabetes, including fungal infections, pain and inflammation [26].

Cryptolepine and its hydrochloride salt possess a range of well-documented biological activities, including antimicrobial, antibacterial, antiinflammatory, antihypertensive, antipyretic, antimuscarinic, antithrombotic, noradrenergic receptor antagonistic and vasodilative properties as well as being used as an effective antimalarial agent [26]. Both the dichloromethane and hot water extracts of the ground roots of *C. sanguinolenta* demonstrated the ability to lower blood glucose in a non-insulin-dependent diabetes mellitus mouse model. *In vivo*-guided fractionation, using the same model resulted in the isolation of cryptolepine as the active ingredient [26, 27]. A small study was conducted using 20 women newly diagnosed with type II diabetes. Plasma glucose levels decreased immediately after administration of the plant extract (ground roots boiled in water, 20mL of extract given four times a day, equivalent to 0.11 mg/kg body weight/day). Mean glucose level of 16.6 mmol/L reduced to 4 mmol/L) [27]. Such studies demonstrate the possibilities of using the ethnomedicinal approach, both in verifying the use of a particular plant to treat a particular disease, but also in the isolation of the active ingredient. Haddad *et al.* [28] evaluated plants traditionally used by the Cree Indians of Canada's Eastern James Bay for treating type II diabetes and its related symptoms. Their investigation found good correlation between those plants highly rated by healers and those showing good activity (glucose lowering, low toxicity and minimal complications) over a range of *in vitro* and *in vivo* tests.

One of the greatest health problems in Africa today is malaria. The increase in drug resistant strains of malaria and the limited number of affordable chemoprophylactic or chemotherapeutic agents makes the development of new antimalarial agents of significant importance. The most commonly used antimalarial drugs are of plant origin or are derived from compounds of plant origin (the quinoline-based alkaloids and artemisinin and its derivatives) [29]. Of the 700 taxa used to treat malaria and/or fever, 134 were selected using weighted criteria (the taxon's association with malaria, documented antiplasmodial potential of the plant family, its use by traditional healers,

whether it occurred in a malaria-endemic area and its popularity in the local plant markets). This resulted in 49% of the plant extracts tested showing good activity (IC_{50}< or = 10μg/mL) and 17% being highly active (IC_{50}< or = 5μg/mL) [29].

A further application of the ethnomedicinal approach is to make cross-cultural comparisons of plant families or genera used for various diseases. Saslis-Lagoudakis *et al*. [30] compared the literature available on plant use for medicinal purposes across three distinct regions; Nepal, New Zealand and the Cape of South Africa. Regressional and binomial analyses were performed at a family level and resulted in the identification of several 'hot' families (Anacardiaceae, Asteraceae, Convolvulaceae, Clusiaceae, Cucurbitaceae, Euphorbiaceae, Geraniaceae, Lamiaceae, Malvaceae, Rubiaceae, Sapindaceae, Sapotaceae and Solanaceae). In spite of many significant differences found across these three regions, the similarities may serve to indicate an underlying biological activity in the commonly used families.

The prevention of chronic non-communicable diseases (like cancer, heart disease, Alzheimer's, cataracts) remains an important area of research. Tan *et al.* have used an ethnobotanical approach in the study of native Australian edible plants [31]. The significant decline in the health of the Aboriginal people has been attributed to dietary changes and the study of a number of their important foods has shown that they do possess some significant health benefits. Some examples are wattle seeds (*Acacia victoriae*), which have shown strong anticancer, anti-inflammatory and anti-oxidant activity in animal models and the Illawarra plum fruit (*Podocarpus elatus*), which has shown anti-oxidant, and pro-apoptotic anti-cancer activity as well as the ability to reduce obesity in a mouse model. Ethnobotanical data can provide useful information about the health enhancing as well as the disease preventing possibilities of traditionally used plants.

The use of an ethnomedicinal approach combined with other tools can enhance the potential for success. Bernard *et al*. [32] have shown that combining an ethnopharmacological with a bioinformatic approach can produce good results. An ethnobotanical study of the Santarem region of Brazil identified 56 plants used locally as anti-inflammatories. Plants found to be active on phospholipase A_2 (an enzyme

which plays a critical role in both the cyclooxygenase and 5-lipoxygenase inflammation pathways), were analysed using the NAPRALERT database and the Combined Chemical Library database to determine the known chemical constituents. Betulinic acid was found to be common and in addition could be inserted into the phospholipase A_2 binding site with the correct energy values (-90kcal/mol). Betulinic acid as well as betulin both showed good activity on phospholipase A_2, as predicted by the bioinformatics information.

The success of the ethnomedicinal approach can sometimes rest on the effectiveness of the extraction and testing methods employed. Supercritical fluid extraction techniques can be tuned to allow for specific compound types to be selectively extracted, allowing for reduced clean up time. Stenholm *et al.* [33] and Wang *et al.* [34] have demonstrated that supercritical fluid extraction can be tuned to extract previously determined bioactive components. Stenholm *et al.* successfully preferentially extracted the COX-2 inhibitory substances α-linolenic acid, ursolic acid and oleanolic acid from *Plantago major* L. while Wang *et al.* developed standard operating procedures for the extraction and identification of dibenzo[*a,c*]cyclooctadiene lignans from *Schisandra chinensis* Baill. These are well known for their hepatoprotective, antioxidant and anticancer activity [34]. Supercritical fluid extraction was successfully used by Sewram *et al.* [35-37] to effectively extract and identify the active components of three medicinally used South African plants. *Clivia miniata* L., *Grewia occidentalis* L. and *Ekebergia capensis* Sparrm. are all used to facilitate labour in pregnant women. Each was extracted using water modified supercritical carbon dioxide and the extracts were found to exhibit uterotonic activity. By coupling a biological detector to the supercritical fluid extractor, active fractions could be immediately detected and active components could be isolated. In the case of *Grewia occidentalis*, the extraction vessel was coupled directly to the bioassay being used (a muscle bath with guinea pig uterine muscle attached to a recorder). The sample was extracted sequentially at three different pressures, 200, 300 and 400 atmospheres, with the 300 atmosphere extract immediately being identified as the most active [36]. The development of techniques that can be used to selectively extract specific compounds of interest are of huge

benefit to ethnomedicinal plant studies and have the potential to increase the rate at which potentially interesting plants can be studied.

The ecological approach

Another non-random method of plant selection involves various ecologically based approaches. Different authors have considered different aspects of the ecological argument, including the relationship between biodiversity and chemodiversity [8], the apparency theory [38, 39], the life-strategy theory [40], chemical defenses and herbivory [41], animal behavior [42] and phylogenetic trends [43, 44].

Ramesha *et al*. [8] have considered the relationship between biodiversity and chemodiversity. Two models were suggested. Firstly that chemodiversity increases linearly with biodiversity: that is that with an increase in biological diversity among taxa in question, the chemical diversity would also increase. Secondly that initially a linear relationship is seen between chemical and biological diversity, but that a leveling off occurs. This would imply that the number of chemical structures is finite. An example of this would be that with all the diversity in alkaloids, only 22 different scaffolds have been identified within the family Asteraceae, with the highest species diversity (21 000) producing only 14 of the 22. The Fabaceae with 16 400 species, produces 17 of the identified 22 scaffolds. The first model would suggest that bioprospecting in the widest possible way should lead to new bioactive chemical entities, while the second model would suggest that specific phylogenetic groups should be studied to enhance the probability of isolating a particular compound-type of interest [8].

Zhu *et al*. [43] conducted a phylogenic study of the naturally-derived drugs approved between 1991 and 2010. It was found that drug-productive species were clustered in specific phylogenic space. It was suggested that a focus on untapped species belonging to already drug-productive families and clusters would increase the likelihood of discovering new useful bioactive compounds. Saslis-Lagoudakis *et al*. [44] have linked ethnomedicinal use of the plants belonging to the genus *Pterocarpus* with specific nodes in the phylogeny. They showed that species used to treat specific diseases, like malaria, tend to be phylogenetically clumped. It could be assumed that

closely related species would share a similar biochemistry and an understanding of phylogenetic relationships between plant species could facilitate plant selection for study.

In order to defend themselves against herbivorous attack, plants have adopted a number of different strategies. The apparency theory divides plants into two groups; those with a short life-cycle (herbs, annuals) that need to invest in small amounts of toxic chemicals to defend themselves (non-apparent) and those with a long life-cycle (apparent) who invest in quantitative, less toxic chemicals to reduce their digestibility [38]. This would suggest that traditional practitioners, as well as those prospecting for new bioactive compounds, should prefer non-apparent plants. de Almeida *et al*. [38] [39] investigated whether the apparency theory could predict traditional medicinal practitioners' choice of plants in the Caatinga and Atlantic Forest regions of northeast Brazil. Neither habitat nor life-strategy were good predictors of phytochemical composition or plant selection in this region. Albuquerque *et al.*[41] have considered both the apparency theory together with the resource-availability hypothesis. In a resource-rich area, plants tend to be fast growing and tend to invest in rapid growth rather than in chemical defense. Plants in resource-poor areas would invest more in chemical defenses and, as such, this makes these plant better candidates for investigation for biological activity. Again the semi-arid Caatinga and the Atlantic Forest regions of Brazil were investigated. They found that neither the apparency theory nor the resource availability hypothesis influenced the choice of plants by traditional medical practitioners. Coley *et al*. [40] compared the defense strategies of mature and young leaves of plant collected throughout the protected wildlands of Panama. They postulated that the young leaves would primarily employ chemical defense systems while the older, tougher leaves would to a lesser degree. Extracts of the fresh leaves were assessed using six *in vitro* bioassays (three cancer cell lines: breast MCF-7, lung H-460 and CNS SF-268; HIV and three tropical disease cell lines: *Leishmania mexicana*, *Plasmodium falciparum* and Chaga's disease). The young leaves showed greater activity than the mature leaves in almost all the bioassays used. In a survey of 18 Panamanian woody species, 10 out of 18 species contained alkaloids only in the young leaves; only 3 species had unique mature leaf alkaloids. It was further postulated that the mature leaves of slow growing shade-tolerant species should display better chemical defenses than those fast growing

species found in better-lit conditions. The cost of replacing leaves in the resource poorer shady conditions would be greater. The activity of these shade-tolerant mature leaves was comparable to the young leaves of the sun-loving plants. In addition a strong phylogenetic signature was observed with some clades being more active than others.

The observation of animal behaviour can sometimes provide insight into potential plant activity. *Khaya* species are endemic to Africa and Madagascar. Local people use the bitter bark and seeds to treat fevers and febrile conditions as well as microbial and worm infestations. Baboons and chimpanzees in Western Uganda have been seen to eat the bark and seeds, which have no nutritional value and are bitter in taste [42]. The petroleum extract of *Khaya anthotheca* showed good activity against *Plasmodium falciparum* K1 (IC_{50} = 0.955μg/mL) and *Trypanosoma brucei rhodesiense* STIB 900 (IC_{50} = 5.72μg/mL). It would appear that in addition to there being evidence for the effectiveness of these plants as used by traditional healers, that the chimpanzees and baboons were using the seeds and bark to self-medicate [42].

Metabolomics

Metabolomics is the study of the 'global metabolite profile' in a system under a given set of conditions' [45]. While metabolomics does not help with the initial choice of plant to be studied, it can help with the rapid analysis of a plant extract and can give a good indication of the active constituents present in a particular extract. Metabolomics allows for the profiling of the entire metabolome or of specific fractions thereof before testing for biological activity. The concentrations and characteristics of the chemical constituents can be determined and statistical analysis (principal component analysis or partial least squares regression analysis) used to link compounds with physiological activity [46]. A problem commonly encountered with bioassay-guided fractionation is the loss of activity during fractionation. An extract may contain a large amount of only moderately active compounds and so appear to be very active or it may contain very small amounts of highly active compounds. Synergistic interactions also need to be considered under such circumstances [47]. The aim of metabolomics is to assess a complex mixture and in so doing allow for the

identification of biologically active constituents without first isolating the compound involved [47].

Metabolomics has proved useful in the analysis of the active constituents of herbal medicines. For example, Iino *et al*. [48] have used capillary electrophoresis time of flight mass spectrometry (CE-TOFMS) to analyse *Toki-Shakuyaku-San*, a Chinese medicine used for gynaecological and obstetric conditions. They were able to identify 737 ± 183.1 (average± standard deviation) metabolite-derived features, of which 119 metabolites were identified [48]. The strength of the metabolomic approach when studying herbal medicines, is that it allows for the identification of all the compounds contributing to the medicinal effect of the plant and takes into account any synergism involved [49].

Albrecht *et al.* [50] have used liquid chromatography mass spectrometry (LC-MS) followed by principal component analysis and orthogonal least squares discriminant analysis to profile and distinguish between *Sutherlandia frutescens* plants growing in different regions of South Africa. Clear differences in the metabolite profile were seen with implications for those in the herbal products industry utilizing *Sutherlandia frutescens* [50].

Computational methods: virtual screening and reverse pharmacognosy

Virtual screening (VS) makes use of the availability of large compound libraries generated by combinatorial and high throughput chemistry to select a small number of likely candidates for experimental testing [51]. Virtual screening can follow one or both of two general strategies: ligand-based virtual screening or structure-based virtual screening [51]. Ligand-based VS makes use of structural and biological activity information from known active compounds to select likely compounds for further testing. Structure-based VS relies on knowledge of the 3-D structure of a molecule and uses techniques such as molecular docking (finding the best position and orientation of a compound within a binding site) to determine possible activity. With sufficient information about the activity and the 3-D structure of a compound, both techniques can be used and the likelihood of a hit increases substantially [51]. The effectiveness of VS can sometimes be compromised by the number of

approximations and assumptions that need to be made but still in some cases substantially (100-fold to 1000-fold) higher hit rates than high throughput screening have been noted [51]. Virtual screening techniques transform chemical databases from storage banks of compounds to useful tools in the drug discovery process and allow all the work already done and recorded to be fully exploited [52].

Decades of work in the field of pharmacognosy have resulted in a vast library of natural products, many of which have undergone only limited testing. Reverse pharmacognosy aims to find new biological targets for natural products by either virtual or real screening and then to link these findings to either the original or another potential plant source [53]. This inverse method starts with the molecule and ends with the plant source. This technique allows for the discovery of new biological activities from known natural compounds [54]. If a compound, available in large quantities from a renewable plant source is found to be active, both the use of the plant by local peoples is verified as well as the potential exploitation of the plant as a resource for development in a local community could be realized [55]. The research team at Greenpharma (France) have demonstrated the success of this technique with compounds like ε-viniferin (anti-inflammatory properties) [53], Merazin (COX-1, COX-2 and PPARγ activity) [55] and honokiol (modulation of testosterone levels) [56] and the details of the technique have been well reviewed [54].

Conclusion

All of the approaches discussed above have shown some success in the identification of plants containing biologically active compounds. This success has not often led to the full development of a new commercial drug. The improvement in high throughput screening techniques and the use of virtual screening methods to identify potential hits should be exploited fully to increase the likelihood of success. Good collaboration between botanists, local traditional healers, chemists and pharmaceutical companies to ensure that potential hits are fully exploited is essential for progress. The high cost of drug development from natural sources necessitates clever approaches in the initial discovery phase. Utilizing a range of non-random approaches to plant choice can facilitate this, as can quicker and more effective

methods of screening for activity. A fuller application of the work already done in natural products, *via* processes like reverse pharmacognosy, can help to open up further avenues of potential success.

Conflict of interests

The authors declare no conflict of interests.

References

[1] *Cragg GM, Newman DJ, Snader KM.* Natural products in drug discovery and developement. J Nat Prod 1997; 60: 52-60

[2] *Newman DJ, Cragg GM, Snader KM.* Natural products as sources of new drugs over the period 1981-2002. J Nat Prod 2003; 66: 1022-1037

[3] *Newman DJ, Cragg GM.* Natural products as sources of new drugs over the last 25 years. J Nat Prod 2007; 70: 461-477

[4] *Newman DJ, Cragg GM.* Natural products as sources of new drugs over 30 years from 1981 to 2010. J Nat Prod 2012; 75: 311-335

[5] *Munos B*. Lessons from 60 years of pharmaceutical innovation. Nat Rev Drug Discov 2009; 8: 959-968

[6] *Firn RD.* Bioprospecting - why is it so unrewarding? Biodiversity and Conservation 2003; 12: 207-216

[7] *Montaser R, Luesch H.* Marine natural products: a new wave of drugs? Future medicinal Chemistry 2011; 3: 1475-1489

[8] *Ramesha BT, Gertsch J, Ravikanth G, Priti V, Ganeshaiah KN, Shaanker RU.* Biodiversity and chemodiversity: future perspectives in bioprospecting. Current Drug Targets 2011; 12: 1515-1530

[9] *Phillipson JD.* Phytochemistry and medicinal plants. Phytochemisry 2001; 56: 237-243

[10] *Heinrich M, Bremner P.* Ethnobotany and ethnopharmacy - their role for anti-cancer drug developement. Current Drug Targets 2006; 7: 239-245

[11] *Lewis WH.* Pharmaceutical discoveries based on ethnomedicinal plants: 1985 to 2000 and beyond. Economic Botany 2003; 57: 126-134

[12] *Baker JT, Borris RP, Carte B, Cordell GA, Soejarto DD, Cragg GM, Gupta MP, Iwu MW, Madulid DR, Tyer VE.* Natural product drug discovery and development: new perspectives on international collaboration. J Nat Prod 1995; 58: 1325-1357

[13] *Heinrich M, Edwards S, Moerman DE, Leonti M.* Ethnopharmacological field studies: a critical assessment of their conceptual basis and methods. J Ethnopharmacol 2009; 124: 1-17

[14] *DeNatale A, Pezzatti GB, Pollio A.* Extending the temporal context of ethnobotanical databases: the case study of the Campania region (Southern Italy). Journal of Ethnobiology and Ethnomedicine 2009; 5: 7 doi: 10.1186/1746-4269-5-7

[15] *Giorgetti M, Negri G, Rodrigues E.* Brazilian plants with possible action on the central nervous system - a study of historical sources from the 16th to 19th century. J Ethnopharmacol 2007; 109: 338-347
[16] *Perry EK, Pickering AT, Wang WW, Houghton P, Perry NSL.* Medicinal Plants and Alzheimer's Disease: Integrating Ethnobotanical and Contemporary Scientific Evidence. The Journal of Alternative and Complementary Medicine 1998; 4: 419-428
[17] *Karunamoorthi K, Husen E*. Knowledge and self-reported practice of thelLocal inhabitants on traditional insect repellent plants in Western Hararghe zone, Ethiopia. J Ethnopharmacol 2012; 141: 212-219
[18] *Lehman AD, Dunkel FV, Klein RA, Ouattara S, Diallo D, Gamby KT, N'Diaye M.* Insect management products from Malian traditional medicine - establishing systematic criteria for their identification. J Ethnopharmacol 2007; 110: 235-249
[19] *Hutchings A, Scott AH, Lewis G, Cunningham A*. Zulu medicinal plants: an inventory1996: University of Kwazulu-Natal Press.
[20] *Douwes E, Crouch NR, Edwards TJ, Mulholland DA.* Regression analysis of southern African ethnomedicinal plants: informing the targeted selection of bioprospecting and pharmacological screening subjects. J Ethnopharmacol 2008; 119: 356-364
[21] *Bennett BC, Husby CE.* Patterns of medicinal plant use: an examination of the Ecuadorian Shuar medicinal flora using contingenct table and binomial analysis. J Ethnopharmacol 2008; 116: 422-430
[22] *Weckerle CS, Cabras S, Castellanos ME, Leonti M.* Quantitative methods in ethnobotany and ethnopharmacology: considering the overall flora - hypothesis testing for over- and underused plant families with the Bayesian approach. J Ethnopharmacol 2011; 137: 837-843
[23] *Weckerle CS, Cabras S, Castellanos ME, Leonti M.* An imprecise probability approach for the detection of over- and underused taxonomic groups with the Campania (Itay) and the Sierra Popoluca (Mexico) medicinal flora. J Ethnopharmacol 2012; 142: 259-264
[24] *Khafagi IK, Dewedar A.* The efficiency of random versus ethno-directed research in the evaluation of Sinai medicinal plants for bioactive compounds. J Ethnopharmacol 2000; 71: 365-376
[25] *Gyllenhaal C, Kadushin MR, Southavong B, Sydara K, Bouamanivong S, Xaiveu M, Xuan LT, Hiep NT, Hung NV, Loc PK, Dac LX, Bich TQ, Cuong N, Ly HM, Zhang HJ, Franzblau SG, Xie H, Riley MC, Elkington BG, Nguyen HT, Waller DP, Ma CY, Tamez P, Tan GT, Pezzuto JM, Soejarto DD.* Ethnobotanical approach versus random approach in the search for new bioactive compounds: support of a hypothesis. Pharmaceutical Biology 2012; 50: 30-41
[26] *Bierer DE, Fort DM, Mendez CD, Luo J, Imbach PA, Dubenko LG, Jolad SD, Gerber RE, Litvak J, Lu Q, Zhang P, Reed MJ, Waldeck N, Bruening RC, Noamesi BK, Hector RF, Carlson TJ, King SR.* Ethnobotanical -directed dicovery of the antihyperglycemic properties of Cryptolepine: its isolation from *Cryptolepsis sanguinolenta*, synthesis and its *in vitro* and *in vivo* activities. J Med Chem 1998; 41: 894-901
[27] *Luo J, Fort DM, Carlson TJ, Noamesi BK, nii-Amon-Kotei D, King SR, Tsai J, Quan J, Hobensack C, Lapresca P, Waldeck N, Mendez CD, Jolad SD, Bierer DE, Reaven GM. Cryptolepsis sanguinolenta:* an ethnobotanical approach to drug discovery and

the isolation of a potentially useful new antihyperglycaemic agent. Diabet Med 1998; 15: 367-374
[28] *Haddad PS, Musallam L, Martineau LC, Harris C, Lavoie L, Arnason JT, Forster B, Bennett S, Johns T, Cuerrier A, Come EC, Come RC, Diamond J, Etapp L, Etapp C, George J, Swallow CH, Swalow JH, Jolly M, Kawapit A, Mamianskum E, Petawabano J, Petawabano S, Petawabano L, Weistche A, Badawi A.* Comprehensive evidence-based assessment and prioritization of potential antidiabetic medicinal plants: a case study from Canadian Eastern James Bay Cree traditional medicine. Evidence-Based Complementary and Alternative Medicine 2012; 1-14
[29] *Clarkson C, Maharaj VJ, Crouch NR, Grace OM, Pillay P, Matsabisa MG, Bhagwandin N, Smith PJ, Folb PI. In vitro* antiplasmodial activity of medicinal plants native to or naturalised in South Africa. J Ethnopharmacol 2004; 92: 177-191
[30] *Saslis-Lagoudakis CH, Williamson EM, Savolainen V, Hawkins JA.* Cross-cultural comparison of three medicinal floras and implications for bioprospecting strategies. J Ethnopharmacol 2011; 135: 476-487
[31] *Tan AC, Konczak I, Sze DM-Y, Ramzan I.* Towards the discovery of novel phytochemicals for disease prevention from native Sustralian plants: an ethnobotanical approach. Asia Pacific Journal of Clinical Nutrition 2010; 19: 330-334
[32] *Bernard P, Scior T, Didier B, Hibert M, Berthon J-Y.* Ethnopharmacology and bioinformatic combination for leads discovery: application to phospholipase A2 inhibitors. Phytochmistry 2001; 58: 865-874
[33] *Stenholm Å, Göransson U, Bohlin L.* Bioassay-guided supercritical fluid extraction of cyclooxygenase-2 inhibiting substances in *Plantago major* L. Phytochemical Analysis 2012; 24: 176-183
[34] *Wang MC, Lai YC, Chang CL.* High throughput screening and antioxidant assay of dibenzo[a,c]cyclooctadiene lignans in modified ultrasonic and supercritical fluid extracts of *Schisandra chinensis* Baill by liquid chromatography-mass spectroscopy and free radical-scavenging method. Journal of Separation Science 2008; 31: 1322-1332
[35] *Sewram V, Raynor MW, Mulholland DA, Raidoo DM.* The uterotonic activity of compounds isolated from the supercritical fluid extract of *Ekerbergia capensis*. J Pharm Biomed Anal 2000; 24: 133-145
[36] *Mulholland DA, Sewram V, Raynor MW, Thornell K, Raidoo DM.* Coupling SFE to uterotonic bioassay: an online investigation of the utertonic activity of compounds from *Grewia occidentalis* (Tiliaceae). South African Journal of Botany 2002; 68: 68-71
[37] *Sewram V, Raynor MW, Mulholland DA, Raidoo DM.* Supercritical fluid extraction and analysis of compounds from *Clivia miniata* for utertonic activity. Planta Med 2001; 67: 451-455
[38] *de Almeida CFCBR, de Amorim ELC, de Albuquerque UP.* Insights into search for new drugs from traditional knowledge: an ethnobotanical and chemical-ecological perspective. Pharmaceutical Biology 2011; 49: 864-873
[39] *de Almeida CFCBR, d eLima-e-Silva TC, d eAmorim ELC, Maia MBd, de Albuquerque UP.* Life strategy and chemical composition as predictors of the selection of medicinal plants from the Caatinga. Journal of Arid Environments 2005; 62: 127-142

[40] *Coley PD, Heller MV, Aizprua R, Araúz B, Flores N, Correa M, Gupta M, Solis PN, Ortega-Barría E, Romero LI, Gómez B, Ramos M, Cubilla-Rios L, Capson TL, Kursar TA.* Using ecological criteria to design plant collection strategies for drug discovery. Frontiers in Ecology and the Environment 2003; 1: 421-428
[41] *Albuquerque UP, Ramos MA, Melo JG.* New strategies for drug discovery in tropical forests based on ethnobotanical and chemical ecological studies. J Ethnopharmacol 2012; 140: 197-201
[42] *Obbo CJD, Makanga B, Mulholland DA, Coombes PH, Brun R.* Antiprotozoal activity of *Khaya anthotheca* (Welv.) C.D.C. a plant used by chimpanzees for self medication. J Ethnopharmacol 2013; 147: 220-223
[43] *Zhu F, ma XH, Qin C, Tao L, Liuy X, Shi Z, Zhang CL, tan CY, Chen YZ, Jiang YY.* Drug discovery prospect from untapped species: indicators from approved natural product drugs. PLoS ONE 2012; 7: 10.1371/journal.pone.0039782
[44] *Saslis-Lagoudakis CH, Klitgaard BB, Forest F, Francis L, Savolainen V, Williamson EM, Hawkins JA.* The use of phylogeny to interpret cross-cultural paterns in plant use and guide medicinal plant discovery: an example from *Pterocarpus*. PLoS ONE 2011; 6: 10.137/journal.pone.0022275
[45] *Rochfort S.* Metabolomics reviewed: a new "omics" platform technology for systems biology and implications for natural products research. J Nat Prod 2005; 68: 1813-1820
[46] *Wyzgoski FJ, Paudel L, Rinaldi PL, Reese RN, Ozgen M, A Z Tulio J, Miller AR, Scheerens JC, Hardy JK.* Modelling relationships among active components in black raspbery (*Rubus occidentalis*) fruit extracts using high-resolution 1-H NMR spectroscopy and multivariate statistical analysis. Journal of Agriculture and Food Chemistry 2010; 58: 3407-3414
[47] *Inui T, Wang Y, Pro SM, Franzblau SG, Pauli GF.* Unbiased evaluation of bioactive secondary metabolites in complex matrices. Fitoterapia 2012; 83: 1218-1225
[48] *Iino K, Sugimoto M, Soga T, Tomita M.* Profiling of the charged metabolites of traditional herbal medicines using capillary electrophoresis time-of-flight mas spectroscopy. Metabolomics 2012; 8: 99-108
[49] *Heinrich M.* Ethnopharmacy and natural product research - multidisciplinary opportunities for research in the metabolomic age. Phytochemistry Letters 2008; 1: 1-5
[50] *Albrecht CF, Stander MA, Grobbelaar MC, Colling J, Kossmann J, Hills PN, Makunga NP.* LC-MS-based metabolomics assists with quality assessment and traceability of wild and cultivated plants of *Sutherlandia frutescens* (Fabaceae). South African Journal of Botany 2012; 82: 33-45
[51] *López-Vallejo F, Caulfield T, Martínez-Mayorga K, Giulianotti MA, Nefzi A, Houghten RA, Medina-Franco JL.* Integrating virtual screening and combinatorial chemistry for accelerated drug discovery. Combinatorial Chemistry and High Throughput Screening 2011; 14: 475-487
[52] *Scior T, Bernard P, Medina-Franco JL, Maggiora GM.* Large compound databases for structure-activity relationships studies in drug discovery. Mini-Reviews in Medicinal Chemistry 2007; 7: 851-860
[53] *Do QT, Renimel I, Andre P, Lugnier C, Muller CD, Bernard P.* Reverse pharmacognosy: application of Selnergy, a new tool for lead discovery. The example of ε -viniferin. Current Drug Discovery Technologies 2005; 2: 1-7

[54] *Blondeau S, Do QT, Scior T, Bernard P, Morin-Allory L.* Reverse pharmacognosy: another way to harness the generosity of nature. Curr Pharm Des 2010; 16: 1682-1696

[55] *Do Q-T, Lamy C, Renimel I, Sauvan N, André P, Himbert F, Morin-Allory L, Bernard P.* Reverse pharmacognosy: identifying biological properties for plants by means of ther molecule constituents: application to Meranzin. Planta Med 2007; 73: 1235-1240

[56] *Bernard P, Scior T, Do QT.* Modulating testosterone pathway: a new strategy to tackle male skin aging. Clinical Interventions in Aging 2012; 7: 351-361