Pak. J. Bot., 43: 175-182, Special Issue, December, 2011 (Medicinal Plants: Conservation & Sustainable use)

PHYTOTHERAPY: RESEARCH GAPS IN PAKISTAN

ABDUL GHAFOOR*, SADAR UDDIN SIDDIQUI, SHAKEEL AHMAD JATOI AND MALIK ASHIQ RABBANI

Institute of Agri-Biotechnology and Genetic Resources (IABGR),
National Agricultural Research Centre (NARC), Islamabad.
**E-mail: Ghafoor59pk@yahoo.com*

Abstract

Medicinal and aromatic plants (MAPs) have a long history of traditional use in Pakistan. The country has a unique and diverse herbal flora, but modern use of herbal treatments in health care is limited by many factors, including possessiveness of herbalists, superstitious mythologies, unrealistic understanding, non-consistent diagnostics, non-sharing of herbal treatments formulations, rigidness of methodologies and unethical collaborative schemes. In order to modernize and fully utilize herbal treatments, all stakeholders must work together in a concerted effort. In this manuscript, we propose a plan that outlines the gaps at three levels and provide a model to bridge these gaps. We need to have a precise research model starting from collection, following through production, and ending with medicine formulations for curing ailments. Only with such a model will Pakistan be able to develop new and effective phytotherapies from its own rich flora.

Background information: Medicinal herbs and traditional healthcare play a pivotal role in curing ailments of most poor people, especially in developing countries including Pakistan. The centuries-old practice of using plant resources in traditional medicines, commonly known as "*unani tib*", is still in existence in the rural areas of Pakistan. Medicinal and aromatic plants (MAPs) are associated with the livelihood of the majority of rural people by providing additional income, either to collectors, retailers or users and practitioners. As most of these plants are wild or endemic to particular areas, they are being displaced due to pressure from other food crop species and demography and household structure changes. In addition, loss of agro-biodiversity and farmers' dependence on a few highly selective crops results in narrow food baskets, which has also caused food and nutrition insecurity and poverty in rural and urban communities. Additionally, the dependence on imports for MAPs has also downsized the whole existing cycle of conservation and enhancement of MAPs. Fewer food sources leads to deficiencies in micronutrients, which were otherwise available in minor crops or MAPs and were traditionally used in various recipes, but a recent shift in food habits has increased the demand for food supplements, which are expensive. The MAPs, along with other plant biodiversity, are being depleted due to human activities and natural calamities. Moreover, the traditional knowledge associated with the conservation and use of MAPs is also disappearing at an alarming rate. The traditional knowledge is as old as the history of mankind, but in this part of the world, traditional knowledge has not been documented or verified scientifically and has given rise to superstitions and myths, which are propagated through print as well as electronic media.

The most sensitized slogan by herbalists is the claim of no side effects from their formulations; these claims require thorough scientific clarification and should be discouraged unless otherwise proven. The Pakistan Forest Institute (PFI), Peshawar is the only institute responsible for medicinal plants but only in their role as a non-timber by-product of the forest. The northern areas of Pakistan, Khyber Pakhtunkhwa, Baluchistan and Azad Jammu and Kashmir are the native home of many herbal plants, and herbal medicine is still the mainstay of health care in these areas (Ali *et al*., 2011). Keeping in view the genetic erosion of MAPs, there is a strong urge to not only conserve this precious genetic diversity for future use but also to evaluate the MAPs for traits of economic significance. The Plant Genetic Resources Program (PGRP) has initiated *ex situ* conservation of MAPs genetic resources to ensure that these plants continue in their roles of poverty alleviation, sustaining the environment, and meeting the healthcare needs for the majority of human and livestock populations. Here we discuss the gaps at three levels dealing with information, herbal genetic resources and their commercialization. A plan is outlined that links all the stakeholders with specific activity to bridge the gaps. We also highlight work done by our institute on MAPs.

Herbal health care: Man has always had need of medicines and healing, and perhaps early man learned from animals to exploit herbal resources during pre-history. In the past, it was difficult to explain why different plants affected humans differently, so myths and superstitions were created as explanations. Throughout history, medicinal plants have figured highly in the life of man: they were always of paramount importance in the treatment of diseases for the common people as well as those in power. The 15th century marked the beginning of the spread of knowledge about medicinal plants and medical practices, in the form of compilations called herbals. Over the centuries, humans used plants without rational explanations of their effects, and this mode of use even exists today in some parts of the world. The 19th century witnessed the rapid development of organic chemistry and pharmacology, and researchers determined the active principles and group of principles responsible for a given therapeutic effect. Gradually, traditional healthcare was overtaken by the scientific-based discipline today known as allopathic healthcare. Systematic knowledge of active ingredients allowed targeted application in therapy, and natural substances frequently served as a model for synthetic preparation of new medicines, which enabled modification of drugs for better effects. However, it is important to note that a better therapeutic effect is usually obtained from a combination of active principles naturally present in various plants rather than from a single isolated substance (David *et al*., 2010). The most important active constituents extracted from plants are the alkaloids, glycosides, essential oils, tannins and bitter principles, all products of secondary metabolism in plants. The products of primary metabolism in plants are mainly sugars, fatty oils, proteins and organic acids. In addition, all plants contain amino acids, enzymes, peptides, and vitamins, which by themselves probably have no therapeutic effect but may regulate metabolic activities and increase the efficiency of the therapeutically important principles. The secondary metabolites of plants are mainly responsible for the plant's therapeutic effects.

MAP sources – benefits and risks: More than a thousand plants are considered important as medicinal and aromatic plants, but about 300 species are used world-wide in the pharmaceutical, food, cosmetics and perfumery industries, both for their medicinal substances and as flavoring agents and natural pigments (Silva & Júnior, 2010). The medicinal action of some plant species is determined by their constituents. These affect the condition and function of various organs in the human body, clear up residual symptoms or destroy the cause of the disease, in most cases infectious micro-organisms. They help increase the body's resistance to disease, retard or ease the processes of natural ageing or facilitate the adaptation of the organism to certain conditions.

Alkaloids, a chemically diverse group of organic nitrogen compounds, rank among the most efficient and therapeutically significant plant substances (Chew *et al*., 2011). Generally, they are extremely toxic, though they do not have a marked therapeutic effect in minute quantities. For this reason, plants containing alkaloids were not often used internally in folk medicine but they were used for external applications. Pure, isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents all over the world for their analgesic, antispasmodic and bactericidal effects (Hussain *et al*., 2010). Examples of such alkaloidal plants are *Chelidonium majus* (Greater Celandine), *Colchicum autumnale* (Autumn Crocus), *Datura stramonium* (Jimsonweed) and *Veratrum album* (White Hellebore). Several thousand alkaloids have been characterized, but only several hundred are used in healthcare therapy. In recent years, attention has been focused on alkaloids with anti-tumourous effects, but it is documented that the plants containing alkaloids should never be self-administered (Mazzio & Soliman, 2009). Glycosides are complex organic substances that when hydrolysed (split by the action of water, acid or enzymes), separate into two parts, a sugar (glycone) component and a non-sugar (aglycone) component. Some glycosides have a pronounced physiological action and are poisonous to man (Wink & Schimmer, 2010). These include, for instance, the cardiac glycosides affecting the muscle tissue of the heart found in *Digitalis purpurea* (Foxglove) without which treatment of heart diseases is unthinkable nowadays, and the glycosides found in Adonis (*Adonis aestivalis*) and Lily of the Valley (*Convallaria majalis*). Therapeutic use of glycosides may be for a laxative action [*Frangula alnus*, syn. *Rhamnus frangula* (Alder Buckthorn), *Rheum rhabarbarum* (Medicinal Rhubarb)], an anti-inflammatory effect [*Agrimonia eupatoria* (Agrimony), *Calendula officinalis* (Pot Marigold), *Chamomilla recutita* syn *Matricaria chamomilla* (Scented Mayweed)], or diuretic and anti-septic properties. Flavonoids, flavonolignans and coumarin derivatives have an important effect on inflammatory processes, liver and gall bladder diseases, and some circulatory disorders; plants known to be useful sources are *Hypericum perforatum* (St. John's Wort), *Agrimonia eupatoria* (Agrimony), *Silybum marianum* (Milk Thistle), and *Fagopyrum esculentum* and *F. tataricum* (Buckwheat). Glycosides also include saponins, which produce a soapy foam that readily identifies them. Their expectorant action is extremely useful in reducing inflammation of upper respiratory passages (Anon., 2009).

Essential oils are generally aromatic, volatile, organic compounds of an 'oil-like character'. They contain terpenes, sesquiterpenes and other components and have a wide range of therapeutic uses. Essential oils extracted from *Thymus serpyllum* (Wild Thyme) and *Thymus vulgaris* (Garden Thyme) are used internally for treating infections of the upper respiratory passages, while extracts of *Achillea millefolium* (Yarrow), *Acorus calamus* (Sweet Flag), *Angelica archangelica* (Angelica) and *Foeniculum vulgare* (Fennel) are used to treat infections of the digestive tract. Similarly, infections of the kidneys, bladder and urinary tract are treated with essential oils of *Juniperus macropoda* (Juniper) and *Petroselinum hortense* (Parsley) (Adams *et al*., 2009). Some essential oils are the principal components of various flavouring agents that also have a favourable effect on digestion, *e.g*., *Anethum graveolens* (Dill), *Carum carvi* (Caraway), *Pimpinella anisum* (Anise), and *Foeniculum vulgare* (Fennel). They stimulate the appetite, promote the flow of gastric secretions and bile, and relieve flatulence. Essential oils also play an important role in medicinal and natural cosmetics. Tannins are organic substances of diverse composition with pronounced astringent properties that hasten the healing of wounds and inflamed mucous membranes. Externally, *Alchemilla mollis* (Lady's Mantle), *Agrimonia eupatoria* (Agrimony), *Salvia officinalis* (Garden Sage) and the bark of *Quercus robur* and *Q. petraea* (Oak) are used for treatment of various ulcers, haemorrhoids, frost-bite and burns, and as mouthwashes for treatment of inflammations and periodontal disease. Internally, they are used for treating diarrhoea and biliousness. Bitter principles are organic substances of extremely varied chemical composition (Vernaza *et al*., 2009) and they stimulate the flow of gastric secretions and help improve digestion.

Plants containing active constituents as mentioned above are used in the treatment of various diseases directly or used in the pharmaceutical, cosmetics, perfumery, and food industries. The pharmaceutical industry uses plants with medically effective chemical substances that are either extremely costly to synthesize or that cannot be produced synthetically. The active constituents are isolated from the plants and then used for manufacturing the drugs. As active ingredients are present in small quantities, sometimes in trace amounts, large-scale production of medicinal plants is required. Digoxin, the main ingredient of many medicines used for cardiac treatments, is obtained from the crude version of the drug, which is extracted from the fermented leaves of the *Digitalis purpurea* (Common Foxglove). Digoxin is present in the crude drug in only tenths of one percent, hence many tons of the substance must be processed to obtain required quantities (Leere *et al*., 2009). Active substances used in pharmaceutical preparations for treating liver disorders include flavonolignans from the fruits of *Silybum marianum* (Milk Thistle). In order to obtain the tons of achenes (seeds) necessary for medical use, Milk Thistle is cultivated on vast areas in South America for pharmaceutical use in Europe and America. Organized collection in the wild provides, among other things, the natural substances used in preparation for the treatment of various circulatory disorders, *e.g*., aescin, which is obtained from the fruits of *Aesculus hippocastanum* (Horse Chestnut). Like digoxin, many tons of the raw material are required. The same is true for rutin, obtained from *Styphnolobium japonicum* syn *Sophora japonica* (Pagoda Tree), for which the crude drug is imported from China. Aromatic plants of the Mediterranean region, *e.g*., *Salvia officinalis* (Common sage), are also gathered by organized collectors who ultimately disturb the natural flora. The active substances in the medicinal plants are present in extremely low quantities so there is a need to breed these plants for higher concentrations of active ingredients, which will ultimately save time and labour to boost economic returns to the MAPs producers.

Assessment of traditional knowledge and ethnobotanical information: During the last three decades, a number of ethnobotanical studies have been conducted and reported in the country. Surveys of indigenous herbal flora indicate that Hazara, Malakand, Kurram Agency, Murree Hills, Azad Kashmir, Northern Areas and Baluchistan are rich and potentially suitable for herbal research and development. Some cultivation of herbs has been reported in Punjab, Sindh, Baluchistan, Khyber Pakhtunkwa and Kashmir (Kumar, 2010). More than 350 herbs are being used for healthcare by 60% of the rural population. Among all the herbs, 100 are collected and sold by local hakims (healers), plant collectors and dealers. During ethnobotanical surveys, the following issues have been discussed:

- Pakistan is rich in an herbal flora that is being used in healthcare in traditional medicine. Varying numbers of MAPs have been reported by various researchers, and multiple researchers are engaged in reporting a routine list of herbs along with some traditional knowledge that has never been authenticated for most of the species.
- Raw material used in the herbal industry, including essential and fixed oils, tannins, gums, resins, and active constituents, has ambiguous origins and no clear route of trade from collector to producer and herbalists is available. The total amount of MAPs available in the country does not meet the amount used. The herbal industry mainly depends on huge imports to meet its demand.
- While reviewing ethnobotanical surveys, no disease seems to be unmentioned and many local herbalists claim to cure serious disease including cancer, hepatitis and AIDS, but without proper experimentation and reproducible results.

Elisabetsky (1991) reported socio-political, economical and ethical issues on medicinal plants and urged that MAPs research be conducted with a clear understanding of a native healthcare system, ethnobotanical know-how, development of cost effective phytotherapeutics, and discovery of prototypic drugs. The world market of MAPs is increasing and is expected to reach 3000 billion USD during the year 2015. The main partners in the herbal market are India, China, USA and Japan. In Pakistan, the trade of MAPs is difficult to estimate because most of the trade is not recorded and documented. Increasing global trade of MAPs has created havoc among the local collectors and retailers and has also caused adulteration and underground marketing of MAPs.

Conservation of germplasm

Medicinal plants have been associated with marginal inputs and interest, although most species are still wild and are being collected haphazardly to fetch maximum income; this has resulted rapid genetic erosion of populations. Although Pakistan may be the centre of diversity for various medicinal plants, there had been no effort to collect and preserve medicinal plants in the past. The PGRP of National Agricultural Research Centre, Islamabad, started to collect medicinal herbs during 2001 and has conserved a considerable collection in a limited time (Ahmad *et al*., 2007; Ahmad & Ghafoor, 2007). More than 2000 accessions of various medicinal plant species have been conserved in the genebank for long-term storage. The passport data for the collected material includes accession number, collection number, local name, site of collection, altitude, longitude, latitude, date of collection and sample type. Table 1 presents the germplasm status of major MAPs preserved and evaluated by the researchers during the last five years. Among these, *Nigella sativa* has been extensively investigated by Iqbal *et al*., (2009; 2010). Similarly, *Plantago ovata* and *Sesamum indicum* have been evaluated thoroughly, while *Lallemantia royleana, Foeniculum vulgare* (Fennel) and *Trigonella foenum-graecum* (Fenugreek) were planted extensively at farmers' fields after selecting promising genotypes at PGRP under field conditions. The collected germplasm is being kept both under active and base collection of the PGRP genebank. Germplasm is stored for short duration (5-10 years) at 10°C, medium term (15-20 years) at 5°C and long term (more than 50 years) at –20°C for further study and distribution to other researchers all over the world.

Table 1. Germplasm status of selected herbs in the national genebank at PGRP, IABGR.

Crop	Preserved in the genebank	Evaluated	Number of traits for which evaluated
Nigella sativa	103	47	29
Plantago ovata	119	103	11
Ricinus communis	98	25	8
Trigonella spp.	131	29	6
Lallemantia royleana	70	2	5
Sesamum indicum	257	105	12
Foeniculum vulgare	123	48	4
Mentha spp.	10	10	6
Fagopyrum esculentum	28	19	7
Ocimum basilicum	60	21	5

Evaluation of medicinal and aromatic plants: Among various medicinal crops, *Nigella sativa* (Blackseed), *Plantago ovata*, *Trigonella foenum-graecum*, *Ricinus communis*, and *Foeniculum vulgare* were evaluated for various traits, while most of the others have been multiplied and conserved in the genebank. Thirty accessions of *Plantago ovata* were acquired from Washington State University (WSU), Regional Plant Introduction Station, Pullman, Washington, USA and seventeen were collected from various parts of the country. All the accessions were planted at Cholistan Institute of Desert Studies (CIDS), Baghdad-ul-Jadid, Bahawalpur, where this crop naturally occurs. Expansion of genetic variability in most of the yield characters and some of the vegetative traits (seedling height, number of branches and stem diameter) resulted in an appearance of a sufficient quantity of more productive forms. It was concluded that exotic germplasm could create additional variability to supplement existing cultivars and superior genotypes were selected for future evaluation. Table 2 presents the results on the spectrum of genetic diversity in major MAPs evaluated for highly heritable traits, agronomic traits, and biochemical and molecular markers. It is evident that all the crop species exhibited a high magnitude of genetic diversity for the evaluation of agronomic traits, whereas total seed protein profiling did not explain intra-specific levels of genetic differentiation. DNA-based markers are likely to be highly useful for resolving intra-specific levels of genetic diversity, and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for seed protein may be useful to resolve inter-specific genetic diversity or might be employed for comparison of unknown species with a known standard species.

Table 2. Genetic diversity in selected medicinal plant species for characterization, evaluation, seed protein profiling and RAPD markers.

Crop	Variation due to				Reference
	Characteriza tion	Evaluation	Seed protein profiling	RAPD markers	
Nigella sativa	Low	High	Low	High	Iqbal *et al.*, 2011
Plantago ovata	Medium	High	Medium	-	Ahmad *et al.*, 2007
Ricinus communis	High	High	Low	-	Anonymous, 2010
Sesamum indicum	Low	High	Low	-	Anonymous, 2010
Foeniculum vulgare	Low	High	Low	-	Anonymous, 2010
Mentha spp.	High	High	-	-	Anonymous, 2010
Fagopyrum esculentum	Low	High	Medium	-	Anonymous, 2010
Ocimum basilicum	High	High	Low		Anonymous, 2010

Development of varieties and agronomic trials: *Nigella sativa* is a new crop in Pakistani agriculture and it can be characterized by the limitation that the seed takes 10-20 days to germinate depending upon soil temperature and moisture. During this germination period, the available moisture in the soil can be reduced if temperature is high. Sometimes additional irrigation is required to get good germination. Due to the long period of germination, weeds may emerge earlier than the crop. To avoid the above problems, sowing on ridges is recommended. There has been little research work on breeding to obtain higher yield, which is another limitation. The use of improved production technology and high yielding varieties can give a seed yield of 1500-1800 kg/ha at properly managed land farms. The study on morphological and agronomic characters on 62 accessions collected from local markets and farmers' field and maintained in the PGRP genebank indicated high variability in days to flower, days to maturity, fruit size and plant vigour, all of which indicate there is potential to increase yield.

Use of biochemical evaluation for validation: Electrophoretic patterns, especially based on seed protein profiles, indicate a clear-cut differentiation among various species of same genera (Emre, 2011), hence similar seeds and confusing taxonomic descriptions could likely be resolved through SDS-PAGE analysis in certain species. As a complement to morphological, biochemical, ecological, and genetic information, molecular markers can contribute greatly to the use of genetic diversity through the descriptive information they provide on the structure of gene pools and accessions. The use of Randomly Amplified Polymorphic DNA (RAPD) techniques for germplasm characterization facilitates the conservation and utilization of plant genetic resources, permitting the identification of unique accessions or sources of genetically diverse germplasm (Vieira *et al*., 2001). The ability of this method to distinguish between taxa also has useful implications in botanical quality analysis (Kapteyn & Simon, 2002). The technique was useful for characterization of germplasm in the PGRP genebank, and its ability to sample any portion of the genome of *Nigella sativa* could help to sort and classify accessions into specific groups (Iqbal *et al*., 2011). The RAPDs have also been used to estimate genetic diversity among crop germplasm, for plant breeding, seed testing programs and for tagging agronomical traits (Jianhua *et al*., 1996; Joshi & Nguyen, 1993). Halward *et al*. (1991) used molecular markers in peanut but did not observe significant levels of polymorphism. This could have been due to the material and primers used in these experiments, as the selection of primer in such studies is very important.

The emerging tools of modern biotechnology could be employed for developing MAPs with higher levels of active compounds, and recently many drugs, including insulin, are being produced using recombinant DNA (Johnson, 1983). Intervention of bioreactors for cell culturing has been discussed by Martin *et al*., (2010) and Yang *et al*., (2011). *In vitro* production of medicines could enhance the efficacy of drugs for targeted functions (Trikha *et al*., 2002). Producing valuable natural substances in the test tube at faster rates with purified substances could be the future theme for the drug industry, therefore we need to adapt modern biotechnological tools to assist production of MAPs.

Constraints of MAPs in Pakistan: Medicinal and aromatic plants (MAPs) are potentially important for the healthcare of poor people in the rural areas of Pakistan and during the last two decades, their worth has increased many-fold (Sher & Hussain, 2010). Some of the constraints associated with MAPs include cultivation, processing, marketing and utilization:

- Poor agricultural practices and indiscriminate harvest coupled with poor post-harvest treatment practices. Good agricultural practices, including organic farming and optimum production technology for each plant species, are needed.
- Lack of genetic research for improvement of yield or active compounds and poor propagation methods for asexually propagated MAPs. Collection of diverse germplasm of MAPs followed by evaluation and conservation is required. Systematic evaluation for active compounds and quantification should be linked with the drug industry. Selection for better genotypes coupled with higher yields of active compounds should be a priority.
- Lack of processing techniques leading to low yields and poor quality products along with no quality control protocols. Post-harvest management is critical for most MAPs because some of the active compounds are volatile or sensitive to certain environmental conditions. More research is needed on proper handling protocols to preserve the active compounds.
- Limited infrastructure for herbal medicines and poor manufacturing practices. Marketing of local herbs is seriously limited because there is not much good quality infrastructure for the drug industry.
- Marketing limitations and lack of local markets for primary processed products. Most of the herbs purchased from open markets lack proper labelling and the year of collection. Similarly, the products need to follow strict rules of drug application rather than ambiguous prescriptions based on non-standardized dosages and drug usages.
- Poor utilization of human resources and equipment, especially non-existence of facilities to fabricate equipment locally. Without local industrial infrastructure, no progress in the drug industry is expected. The local drug industry based on MAPs should be strengthened for value addition.
- Possessiveness of herbalists hindering the spread of herbal treatments. Information on proper application of herbal treatments should be shared regionally and globally through scientific publications. Use of heavy metals and steroids in treatments should be restricted and the formulations must accurately list ingredients.
- Superstitious mythologies surrounding herbal treatments. Specific knowledge on method of treatment and effects needs to be developed for herbal treatments and modern diagnostic facilities should be encouraged to accept herbal treatments that have been authenticated. The claims of “no side effects” of herbal prescriptions should be verified scientifically rather than being used as a slogan to promote herbal treatments.
- Unrealistic understanding, non-consistent diagnostics, non-sharing of herbal treatments, rigidness of methodologies. Herbalists should develop a scientific consensus on diagnostics and application of modern diagnostics methods for uniform treatment rather than basing treatment on intuition.
- Unethical collaborative schemes. This is a societal problem that affects both herbal healthcare and scientific research. Dishonesty and intellectual cheating have marred our research, especially on herbal healthcare. Research developments are vulnerable to theft or misappropriation, which has discouraged continued work and improvement on MAPs. Due to the numerous reasons listed above and

others, the development of effective phytotherapy has suffered and cannot be addressed and corrected without concerted efforts by all the stakeholders.

Systematic cultivation of MAPs needs specific cultural practices and agronomical requirements that cannot be accomplished by one or few institutes (Reed *et al*., 2004). These are species-specific and are dependent on soil, water and climatic conditions. Although a range of agro-ecological resources are available in Pakistan, no human resources are available according to the specific requirement for MAPs under specific environmental conditions (Reubens *et al*., 2011). There is a need to establish research groups on particular MAPs with known problems. Research and development work is required to formulate good agricultural practices including appropriate selection and identification of elite material, propagation methods, cultivation techniques, harvesting, stepwise quality control of raw material to processing stages, post-harvest treatment, storage/expiry protocols and safety guidelines.

The proposed scheme for streamlining MAPs through bridging research gaps (BRG) has been outlined in Fig. 1. The research gaps include three broad issues which are further subdivided in three clusters. The cluster consisting of traditional knowledge, ethnobotanical studies and education move together under the guidance of a coordinated forum (the experts from all the stakeholders). Through appropriate methodologies and experimentations, there is a need to compile available information and validation of traditional knowledge through research that will be the only way forward to develop drugs. Unpublished and unsubstantiated formulations should be discouraged to ensure proper healthcare for poor people, especially in rural areas. Collection and conservation has a key role in crop improvement for various traits of economic importance, and this area is the weakest for MAPs. Only a few examples exist for precise conservation of MAPs and most of these are not characterized. Evaluation of MAPs requires special skills and is not the same as the methods used for ordinary grain crops. The active ingredients and the time of collection are important factors that must be worked out in collaboration with universities and research and development institutes.

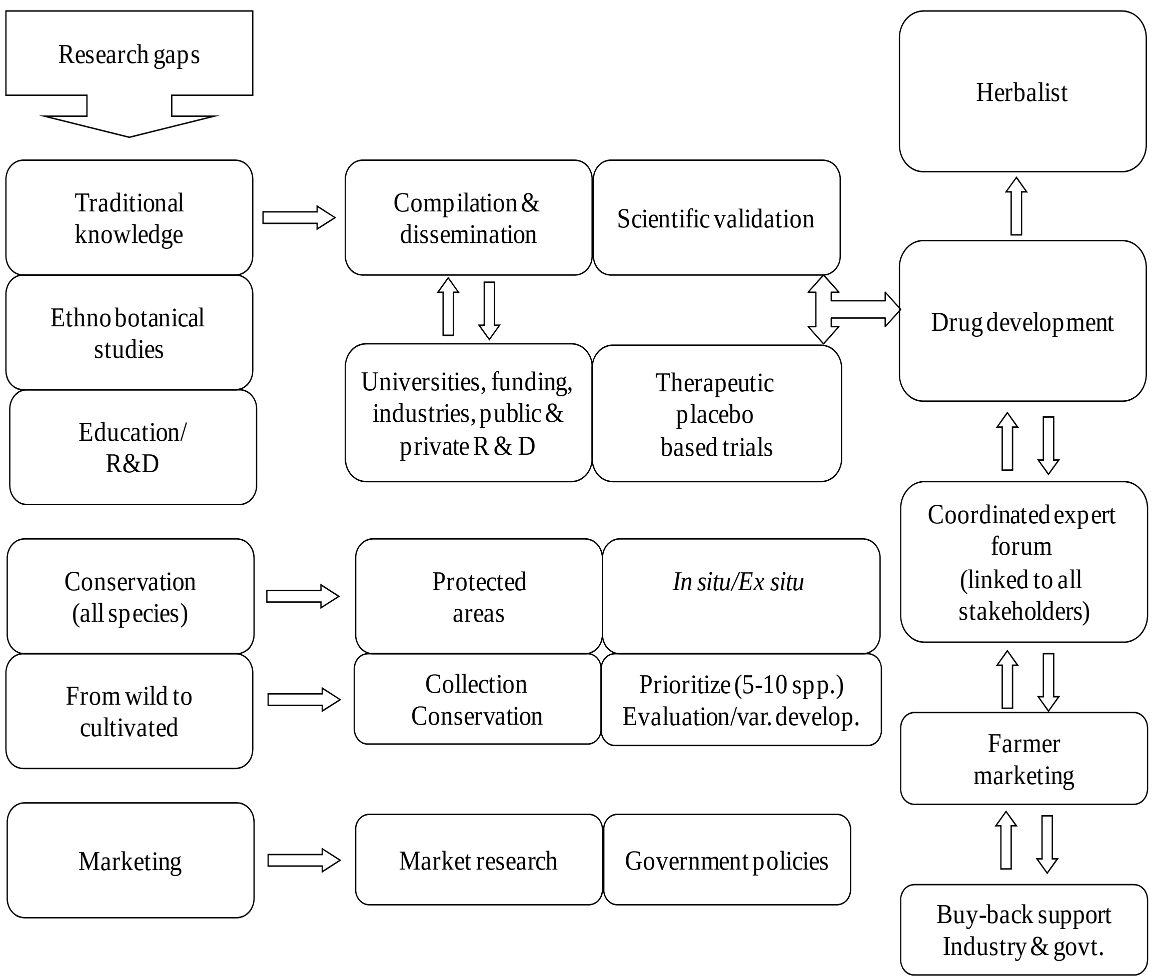

Fig. 1. Strategic plan addressing Bridging Research Gaps (BRG) in Medicinal and Aromatic Plants (MAPs) in Pakistan

Marketing is the most crucial issue in MAPs, especially for cultivated species. Although the herbal industry claims to consume much higher quantities of MAPs than are available in the country, they are reluctant to buy from the local producers unless someone has strong ties with industry. Market research, along with exploring new avenues, should be strengthened and direct purchase from registered growers and a buy-back policy can ensure good production of MAPs. All the stakeholders share the common interest of delivering herbal medicine for health care, therefore one forum must be operative at the federal level to formulate all the activities in a network. The herbalists may only be allowed to market herbal medicine following the rules of the pharmacopeia and drug development authority, with no exceptions. Connections between herbalists and industry or researchers could only enhance their expertise, but as a general rule, one agency must verify what is being marketed for healthcare.

Conclusions

Most developed nations have transformed traditional herbal knowledge into a well organized drug industry based on systematic research and development that has contributed substantially to their economies. This transition from traditional knowledge to modern healthcare use remains out of reach in Pakistan due to non-scientific and unethical behaviour of researchers and herbalists, including poor or no coordination among various stakeholders. Although Pakistan is rich in MAPs diversity, information is fragmented and poorly organized, and more than 20 development projects have been executed with no significant output except for a few seminars/workshops. Local MAPs availability has never been investigated for markets and utilization, and mysterious herbal formulations catalyzed by inorganic substances remain an unsolved issue. Emerging tools of biotechnology are potentially important for future MAPs evaluation, development, utilization and conservation. The proposed BRG scheme outlined in the text is a preliminary effort for streamlining research and development activities in MAPs to ensure the role of herbalists and MAPs in healthcare for the majority of rural people of Pakistan.

References

Adams, M., C. Berset, M. Kessler and M. Hamburger. 2009. Medicinal herbs for the treatment of rheumatic disorders—A survey of European herbals from the 16th and 17th century. *Journal of Ethnopharmacology*, 121(3): 343-359.

Ahmad, Z. and A. Ghafoor. 2007. *Nigella sativa*: *A Potential Commodity Crop Diversification, Traditionally used in Healthcare*. In: Breeding of Neglected and Under-Utilized Crops, Spices and Herbs, Eds: Ochatt, A. and S.M. Jain, Science Publishers, Enfield, NH, USA, pp. 215-230.

Ahmad, Z., A. Ghafoor and M. Arshad. 2007. *Plantago ovata Forssk. - A Crop of Arid and Dry Climates with Immense Herbal and Pharmaceutical importance*. In: *Breeding of Neglected and Under-Utilized Crops, Spices and Herbs*, (Eds.): A. Ochatt and S.M. Jain, Science Publishers, Enfield, NH, USA, pp. 231-249.

Ali, H., J. Sannai, H. Sher and A. Rashid. 2011. Ethnobotanical profile of some plant resources in Malam Jabba valley of Swat, Pakistan. *Journal of Medicinal Plants Research*, 5(17): 4171-4180.

Anonymous. 2009. Inflammation reducing action of synergistic mixture of bisabolo and ginger extracts. United States Patent Application 20090238905.

Anonymous. 2010. Annual Report of Institute of Agri-Biotechnology and Genetic Resources (IABGR), NARC, Islamabad, Pakistan. p. 85.

Chew, Y.L., E.W.L. Chan, P.L. Tan, Y.Y. Lim, J. Stanslas and J.K. Goh. 2011. Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia. *BMC Complementary and Alternative Medicine*, 11: 12.

David M.L., C.S. Patrick, B.P. Lynette, R.G. Michael and D.A. Kinghorn. 2010. Potential of plant-derived natural products in the treatment of leukemia and lymphoma. *Current Drug Targets*, 11(7): 812-822.

Elisabetsky, E. 1991. Sociopolitical, economical and ethical issues in medicinal plant research. *Journal of Ethnopharmacology*, 3: 235-239.

Emre, I. 2011. Determination of genetic diversity in the *Vicia* L. (Section *Vicia*) by using SDS-PAGE. *Pak. J. Bot.*, 43(3): 1429-1432.

Halward, T.M., H.T. Stalker, E.A. Larue and G. Kochert. 1991. Genetic variation detectable with molecular markers among unadapted germplasm resources of cultivated peanut and related wild species. *Genome*, 34: 1013-1020.

Hussain, J., Z. Muhammad, R. Ullah, F.U. Khan, I.U. Khan, N. Khan, J. Ali and S. Jan. 2010. Evaluation of the chemical composition of *Sonchus eruca* and *Sonchus asper. Journal of American Science*, 6(9): 231-235.

Iqbal, M.S., A. Ghafoor, A.S. Qureshi, M.R. Khan and M.I. Chaudhary. 2009. Quantification and diversity in the black seeds (*Nigella sativa* L.) gene stock of Pakistan for their composition of mineral nutrients. *Journal of Chemical Society Pakistan*, 31(5): 793-800.

Iqbal, M.S., A.S. Qureshi and A. Ghafoor. 2010. Evaluation of *Nigella sativa* L., for genetic variation and *ex-situ* conservation. *Pakistan Journal of Botany*, 42(4): 2489-2495.

Iqbal, M.S., S. Nadeem, S. Mehboob, A. Ghafoor, M.I. Rajoka, A.S. Qureshi and B. Niaz. 2011. Exploration of genotype specific fingerprinting of *Nigella sativa* L. using RAPD markers. *Turkish Journal of Agriculture and Forestry*, 35: 569-578.

Jianhua, Z., M.B. McDonald and P.M. Sweeney. 1996. Random amplified polymorphic DNA (RAPDs) from seeds of differing soybean and maize accessions. *Seed Sci. & Tech.*, 24: 513-522.

Johnson, I.S. 1983. Human insulin from recombinant DNA technology. *Science*, 219:632-637.

Joshi, C.P. and H.T. Nguyen. 1993. RAPD (random amplified polymorphic DNA) analysis based on inter-varietal genetic relationship among hexaploid wheat. *Plant Science*, 93: 95-103.

Kapteyn, J., and J.E. Simon. 2002. *The use of RAPDs for assessment of identity, diversity and quality of Echinacea.* In: Trends in new crops and new uses. J Janick and A Whipkey (eds.), ASHSPress, Alexandria, VA. pp. 509-513.

Kumar, R. 2010. *Early history of Jammu region: pre-historic to 6th century*. Gyan Publishing House, 658 p.

Leere, Ø. E., J. Unni, S.O. Mimi, B. Stein and C.A. Solberg. 2009. Determination of digoxin and digitoxin in whole blood. *Journal of Analytical Toxicology*, 33(7): 372-378.

Martin, I., A.R. Stefania and D. Wendt. 2010. Bioreactor systems in regenerative medicine. Advances in regenerative medicine: Role of nanotechnology and engineering principles. *NATO Science for Peace and Security Series A: Chemistry and Biology*, 95-113, DOI: 10.1007/978-90-481-8790-4_6.

Mazzio, E.A. and K.F.A. Soliman. 2009. *In vitro* screening for the tumoricidal properties of international medicinal herbs. *Phytotherapy Research*, 23(3): 385-398.

Reed, B.M., F. Engelmann, M.E. Dulloo and J.M.M. Engels. 2004. Technical guidelines for the management of field and *in vitro* germplasm collections. IPGRI Handbooks for Genebanks No. 7.

Reubens, B., C. Moeremans, J. Poesen, J. Nyssen, S. Tewoldeberhan, S. Franzel, J. Deckers, C. Orwa and B. Muys. 2011. Tree species selection for land rehabilitation in Ethiopia: from fragmented knowledge to an integrated multi-criteria decision approach. *Agroforestry Systems*, 82(3):303-330.

Sher, H. and F. Hussain. 2010. Ethnobotanical evaluation of some plant resources in Northern part of Pakistan. *African Journal of Biotechnology*, 8 (17): 4066-4076.

Silva, N.C.C. and F.A. Júnior. 2010. Biological properties of medicinal plants: a review of their antimicrobial activity. *The Journal of Venomous Animals and Toxins including Tropical Diseases*, 16(3):402-413.

Trikha, M., L. Yan and M.T Nakada. 2002. Monoclonal antibodies as therapeutics in oncology. *Current Opinion in Biotechnology*, 13(6): 609-614.

Vernaza, G., F.C.A.U. Matsuura, Y.K. Chang and C.J. Steel. 2009. Effect of some extrusion variables on residual quantity of cyanogenic compounds in an organic breakfast cereal containing passion fruit fiber. *Cereal Chemistry*, 86(3): 302-306.

Vieira, R.F., R. Grayer, A. Paton and J.E. Simon. 2001. Genetic diversity of *Ocimum gratissimum* L. based on volatile constituents, flavonoids and RAPD markers. *Biochem. Syst. Ecol.*, 29: 287-304.

Wink, M. and O. Schimmer. 2010. *Molecular Modes of Action of Defensive Secondary Metabolites, in Annual Plant Reviews Volume 39*: Functions and Biotechnology of Plant Secondary Metabolites, Second edition (Ed.): M. Wink, Wiley-Blackwell, Oxford, UK. DOI: 10.1002/9781444318876.ch2.

Yang, X., D. Wang, J. Hao, M. Gong, V. Arlet, G. Balian, F.H. Shen and X.J. Li. 2011. Enhancement of matrix production and cell proliferation in human annulus cells under bioreactor culture. *Tissue Engineering Part A*, 17(11-12): 1595-1603. DOI:10.1089/ten.tea.2010.0449.

(Received for publication 26 October 2011)