



Invited Commentary : Open Access

AYUSH drugs need evidence based scientific research

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Abstract

There are almost 1500 medicinal plants used in Ayurveda, Unani, Siddha and Homeopathy (AYUSH) system, among them 25 to 30 plants are popular in terms of well-defined active principles and activity. The medicinal plants provide the raw materials for pharmaceutical, perfumery, flavour and cosmetic industries. About 80% of the world's rural population depends up on native traditional medicine. The plant based traditional remedies are very cheap and without any side effect. However, their pharmacology and estimation of activity HPLC and other methods is restricted to extracts only but not on formulations. "HPLC profile-pattern" of peaks with reproducible retention time, provides ready. Identification-estimation of "active principles called chemical bioactive markers" in a multi-herbal formulation extract. This method called "HPLC finger printing" "biomarker" characterization. So far, these studies are restricted crude plant drugs and extracts. These are not reflected in ready to use formulations. Some suggestions are given at the end. Related issues to make plant based AYUSH drugs popular is also examined in the commentary.

Keywords

AYUSH, Active principles, HPLC, Clinical trials, Limitations

1. Introduction

In India, the earliest reference to curative properties of some plant medicines were mentioned in Rigveda (years 3500-1800 BC). Among the four Vedas, Rigveda, Yajurveda, Samaveda and Atharveda, the last veda is also considered to be precursor of Ayurveda. The term Ayurveda means knowledge of science of life (Ayu) from birth to death. Texts like "Charaksamhita" and "Sushurthasamhita" documented medicinal plants about 1000 years B.C. back.

Apart from Ayurveda, Unani drugs, Homeopathy and Siddha are mostly derived from plant sources. Unanihasits origin in Greece, about 2500 years ago. Subsequently, many medicinal plants from middle and Far East were added in to enrich Unani system. Siddha system is another traditional system which constitutes medicinal plants and bhasmas (metal oxide) found mostly in south India. Siddhars (priests with medicinal knowledge) very enlightened souls, had reputation to treat several diseases. This system is almost identified with the Tamil literature and has its own distinct tradition and cultural heritage. In all the traditional medicines of India, Ayurveda, Unani or Siddha, Homeopathy many plants that are used are almost common. Homeopathy has its origin from German and

Europe, uses dilute alcoholic or water extracts of plants called tinctures. The tinctures are very dilute solutions, more dilutions increase the potency. The prime discoverer of homeopathy is German physician is Samuel Hahnemann during the end of the 18th century.

1.1 Most popular AYUSH drugs

The term AYUSH means Ayurveda, Unani, Siddha and Homeopathy (AYUSH) which is the name of union government health department. Incidentally, AYUSH also means longevity of life. The first three systems of medicines use of plant powders from juices, infusion, decoctions, tea, poultices from fruits, roots, bark, flowers, bark or food, administered with water, honey, milk, honey and jaggery. Some of the ayurvedic products are formulated in self-generated alcohol (fermentation, aristas), ghritas, formulated in ghee and jaggery, *etc.* Homeopathy uses very dilute tinctures, dilute alcoholic or water extracts of plants used as such or absorbed on sugar pills and some salts also. Majority of the plants used in AYUSH are common, but differ in formulations.

With the advent of the development of science, especially chemistry, pharmacology, random clinical developments in medicinal practices, the awareness to understand the active chemical principle responsible to exert medicinal activity, its quantification are necessary to make popular all the four systems of AYUSH. There are considerable advancements in this direction in the recent years. In fact for most of the development of allopathic drugs, natural products used in traditional medicines provides leads or they themselves in their own right became drugs, during the latter half of 1940's. Examples are quinine, reserpine, atropine, colchicin, digoxin, taxol and camptothecin to mention few. To be specific one example, morphine used in extreme pain. Diosgenin is used as a starting material for steroidal drugs such as progesterone, *etc.* Thus, the importance of natural products for discovery of new drugs is not relegated. After 1940's, allopathic drugs (synthetics, antibiotics from fermented strains, modification of plant natural products) made rapid strides into clinical use. Correspondingly, the use of traditional medicine decreased but not relegated. WHO laid importance to traditional medicinal plants by suggesting regulatory guidance such as quality, safety, toxicity and efficacy.

1.2 Popularity of AYUSH drugs alternative medicines/ supplementary medicines

However, the popularity of traditional medicine either in India, Africa, Orient (China *etc.*) or in West (America, Europe) is not reduced 80% of the Indian population in India and other developing countries,

especially urban or rural poor, use traditional medicine either due to belief or non-accessibility of modern-allopathic medicine. Allopathic treatment/hospitals are expensive and not accessible to poor. Therefore, poor has no alternative other than relying on AYUSH, tribal medicine and rural faith healers.

Even urban India also, AYUSH medicines are also popular to treat minor ailments or to manage chronic diseases, rheumatism, diabetes or for rejuvenation. Now-a-days, plant medicines are also used for cancer and AIDS as adjuvant therapy, which are to be used along with allopathic. Toxicity, side effects and cost of allopathic drugs is another reason for the popularity of AYUSH drugs.

Even in developed countries, the popularity of plant medicines is increasing. In America, 15 millions use as per one estimate, and in Denmark 15% of the people, Canada 15% Finland 33% and in Australia 15%. However, they use it as “alternative medicine” or dietary supplements. In the strict sense, alternative is strictly not alternative to allopathic but often they use it concurrently with modern medicine, often called supplementary medicines. The patients may not reveal to modern medicine practitioners that they have been using the present medicines. In India, AYUSH physicians suggest to use along with allopathic, with some precautions for some diseases. The popularity of AYUSH medicines as alternative medicines or dietary supplements in India can be gauged by the numerous advertisements over media, TV, etc. AYUSH products by far will be used for diseases of longer duration, contains beneficial adjuvants (like honey, milk and jaggery), prophylactic, and gives a sense of well-being and supply antioxidants.

1.3 Standardisation of plant medicinal extracts/herbal drugs by HPLC profile, etc

Therefore, there is need to establish the chemical structures responsible for the activity of the plant drugs, standardize the extracts and formulations with respect to their active principles. Such active principles are called “bioactive markers.” These markers are needed to be characterized by analytical tools by conventional techniques as well as by HPLC, HPLC/MS and HPTLC, etc. Along with the bioactive marker, a number of closely related compounds are also

present in the plant and formulations, although in minor amounts. These minor bioactive principles also exert similar activity or act as synergists. These minor bioactive principles could be characterized by HPLC profile or HPTLC profile or finger printing. Thus, quantification or standardization of a plant drug in all the stages, the growth of the plant wild or cultivated, storage, extraction process often hot water, fresh or dried, or alcohol solvent extraction, formulations, and shelf-life studies of formulations is desirable. For this quantification apart from HPLC characterization of bioactive marker(s) gravimetric, volumetric and other analytical studies are necessary. As already pointed out there at least 1500 medicinal plants are in use in traditional medicines, but only about 25 or 30 medicinal plants are popular in terms of as single plant drugs. Herein also, only 30-40 plant drugs reported to have well characterized active principles are well known in India. For examples, formulation of *Rauvolfia serpentina*, used for hypertension contains reserpine as main active principle as marker and other related minor alkaloids which may also similar bioactivity. Therefore, HPLC principle reveals all the bioactive chemicals present in the formulation. Such HPLC profile useful for identification of the *Rauvolfia* formulation as well as estimation of main bioactive markers, reserpine. As such HPLC profile or HPLC finger print is a must to characterise, identify and estimate the active constitute to achieve the reproducibility of the drug action. GC and gravimetric analysis, pharmacological studies were done on many of these plants but restricted to laboratory and processed extract only but not on formulations. This is a big lacuna in AYUSH medicines.

However, standardization of the end formulation, to be used by patients, was carried out hardly on 4 or 5 formulations only. Examples where in identification of the active principle standardization of the formulation were made on drugs from *Boswellia*, *Bacopa*, *Psoralea corylifolia*, *Cassia angustifolia*, *Gymnema* and *Guggul*, etc., (Table 1). In contrast, all the plant drugs that are sold in America are characterized with respect to the active principles. Example *Silybium marianum* extract, 75% total flavonoids with respective active principle silymarin and soya capsules or tablets were standardized with respect to 40% isoflavonoid content in which genestin is the most active isoflavone.

Table 1: Active ingredient, preferable % and activity isolated from MAPs in India

Name of the plant	Active ingredient, preferable % and activity
<i>Andrographis Paniculata</i> (Chao and Lin, 2010)	10-15 % Andrographolide (liver diseases)
<i>Curcuma longa</i> (Wahlstrom and Blennow, 1978)	Essential oils and curcumin (anti-oxidant)
<i>Bacopa monniera</i> (Russo and Borrelli, 2005)	35-38 % Bacoside A & B (to improve memory)
<i>Centella asiatica</i> (European pharmacopoeia, 2005)	10-15 % Asiaticoside and 14-18 % triterpense (to improve memory)
<i>Withania Somnifera</i> (Anonymous monograph, 2004)	1.5- to 2 % Withanolide and 1-3 % alkaloid (immuno modulator)
<i>Fagopyrum esculentum</i> (Buck wheat) extract (Itagaki <i>et al.</i> , 2010)	5 % Rutin (antioxidant)
<i>Bixaorellana</i> (Annato seeds) extract (Bouvier <i>et al.</i> , 2003)	Bixin and norbixin as per application (natural colouring matter)
<i>Trachyspermum ammi</i> (Ajwain) extract (Nagalakshmi <i>et al.</i> , 2000)	Thymol and other essentialoils (digestive)
<i>Phyllanthus amarus</i> extract (Chew and Rodman, 1979)	3% Bitters (hepatoprotective)

Thus, there is need to standardize as many as Indian plant drugs as possible with respect “bioactive marker compounds”. However, for many of the medicinal plants, the active principle is not known or defined even after chemical examination but still some of these are

very popular drugs. Many Ayurvedic formulations are made up from multi herbals. In such cases, where active principle not known also, HPLC profile will be useful to identify or quantify the drug. HPLC profile is also called HPLC ‘finger printing’ where active

principles were chemically defined or not. A good plant formulation with quantified biomarker component may give reproducibility of the activity. By HPLC analysis (profile or finger printing) is the most rapid to characterize or quantify the plant extracts, or formulations. There is a rider that many formulations are patient specific and some are doctor specific, wherein faith and placebo effect predominates.

1.4 Active chemical constituents found in the plant and AYUSH formulations

If, a herb medicinal plant is used for a specific ailment, the active chemical constituent found in the plant, often a secondary metabolite, will be responsible for the activity. The active principle may belong to one of the classes-alkaloid, triterpinoid, often triterpinoid glycosides, steroidal, glycosides, mono-or-sequiterpinoids, cardioglycosides, or polyphenols. The term polyphenols include catechins, proanthocyanidines, anthocyanidines and anthocyanins, tannins, condensed tannins, which are oligomers of catechins, and hydrolysable tannins, which are esters of gallic acid, with sugars usually glucose, ellagic acids and its esters and also ellagitannins, flavonoids, or their glycosides, etc. These polyphenols are important exert antioxidant activity. These entire active products defined as bioactive markers could be readily identified and quantified by HPLC/GC or by any other spectral/conventional analysis. The most rapid and reproducible method is HPLC, GC, HPTLC profile/finger printing of the extractives from plant or in formulations.

For example, *Rauwolfia serpentina* contains the most active hypotensive principle, reserpine, and also the plant elaborates a number of closely related alkaloids in minor amounts. These minor amounts also exert similar activity or act as synergist. The AYUSH formulations contain usually the most bioactive compound (bioactive marker) and also related bioactive compounds in minor amount. All these constituents could be identified by HPLC profile, often termed as HPLC finger printing. Now-a-days many scientists feel this HPLC finger printing is a necessary tool for identifying and quantifying the bioactive principle in formulations as well as plant extract. Even if the active principle is not known, HPLC profile/finger print is still useful to identify the plant constituents in plants. Apart from HPLC, HPLC/MS/MS, GCMS, gravimetric, volumetric, fluorescence spectroscopy also have been in use, in a very limited way. However, such analysis is at the moment is restricted to handful of AYUSH plants only. On the other hand, the formulations found in advanced countries have all the parameters studied before releasing in to the market. Such studies are desirable for as many formulations as possible to gain popularity in India market.

At the moment, if we look at the international scenario, the most popular Indian products are *Cassia angustifolia* and *Plantago ovata*. Some herbal drug manufacturers are exporting a number plant extracts, but it is mostly for experimental and trial purposes, but has not reached to commercial level in those countries. Unfortunately, many herbal dealers canvas that Indian medicinal plants have a lot of demand and encourage farmers to grow the same.

1.5 Traditional methods of extraction, formulation and method of use by patients in AYUSH system

As per the AYUSH principles, traditional methods of isolation (water mostly, solvents like methanol or ethanol in exceptional cases) need to be followed by the manufacturers for formulation, as per the

tenants of the traditional medicine. To make these AYUSH products more popular in India and abroad, the presence of active principle in required amount to exert its activity is to be determined. Therefore, the most important issue before AYUSH scientists, is the estimation of the active principle-bioactive marker compounds, analysis of the total active principles in plants (wild or cultivated), by usual chemical extraction, by AYUSH methods of extraction and in AYUSH formulations is necessary to get reproducibility of the activity of the medicine.

Therefore, it is best to leave the formulation part to the traditional methods, while the AYUSH physicians, scientists and students need to be convinced that formulation should contain active principle (bioactive marker) along with related compounds. The duty of the-chemist is to ensure AYUSH products (include Homeopathy) as well as plant material to be used contain in all stages from raw material to manufacturing stage to formulation stage (includes shelf- life) contain active principle and in required quantity.

Question arises what is the right amount of active products a formulation should contain. Once again, there is no satisfactory answer from AYUSH scientists or physicians. One way to tackle the problem is take the most widely known formulations from different companies (to mention few Brahmi, Aswagandarista, Mahanarayanatilam, Saraswatarista, Madipalarasayanam, Albosang, some liver tonics and antidiabetic products) and study its pharmacology in institutes of AYUSH, medical or pharmacy colleges. Readily verifiable results could be obtained pharmacologically for liver, diabetic (blood sugar reduction), anti-inflammatory activity and for skin diseases. Such results could be correlated to bioactive markers and their quantity in formulations. So, far such pharmacology studies are restricted to plant extracts/crude extracts in pharmacy and medical colleges. On the other hand, in AYUSH colleges/institutes random clinical trials are often restricted to plant extracts but not on to be released commercial formulations.

Bioactive products, pharmacological activity, random clinical trials and patient satisfaction and correlation by statistical analysis provide us to arrive at an ideal formulation for further popularization. To the best of our knowledge, such correlations do not seem to have been made so far. It is one of the investigation need to be carried out by scientists belonging to chemists, pharmacology, medical and AYUSH scientists. Thus, AYUSH commercial formulations lack the above mentioned data like pharmacological, chemical standardization and clinical data.

1.6 Modern methods of formulations of AYUSH products using machinery

Now tribals or cultivators supply the material to an agent who in turn sells it to small scale extractor. The extractor in turn sells to big formulation unit. Some of the few names of the big formulation units are Aryavaidyasala, Kotakkal, Imcomp, Adayar Chennai and Gandhigramayurveda manufacturing unit, Dindigul in Tamilnadu. Dabur, Baidynath, etc. They use machinery, reactors for pulverising, extraction, blending with adjuvants which are a welcome feature. However, most Govt. State units of AYUSH manufacturing units are far from using good manufacturing practices (GMP). It is noticed some AYUSH use bore well water which contains harmful metal salts.

During the process of plant extraction in large scale by extraction units using water or hydroalcoholic extraction, chemical structural changes of the active principle may take place. Such conspicuous examples are a steroidal saponin which has three sugars and may be converted into two sugar saponin or to the genin itself. Yet some molecules suffer isomerisation of double bonds or racemisation at chiral centres. That may or may not alter the bioactivity. One way to answer the problem is phytochemists need to extract the bioactive chemical constituents from the formulations also and verify and quantify the structures of the bioactive principles in the extracts/formulations. It is also necessary to study the pharmacology of the isolated constituents from the formulations again, by phytochemists and pharmacologists.

1.7 AYUSH Pharmacopoeia

Ayurvedic pharmacopoeia, as well as Unani pharmacopoeia is revised from time-to-time. The notable changes in the revision includes colour reactions of the various chemical constituents, names and/or structural class the active constituents if available, percentage of the solid extractives, ash values, pH and in some case gravimetric analysis and TLC profile. It is desirable to include HPLC profile and HPTLC profile of the active products, quantification of bioactive marker compounds. However, these are not found in pharmacopoeia. These are requisite parameters for any plant drugs when it comes to export. It is a big task but a beginning can be made for at least half a dozen formulations/drugs. Accordingly AYUSH drug testing laboratories/inspectors need to be acquainted on these aspects. This is also another requirement to make AYUSH popular.

1.8 Adulteration, contamination-major problems in AYUSH

One problem facing Indian herbal drug Industry is contamination of the herbal formulations with toxic metals like arsenic, lead, mercury and cadmium, *etc.* This problem arises due to agricultural practices like the use of deep bore wells which contaminate water with arsenic, while cultivation in urban areas are responsible for the contamination of other toxic metals like cadmium, lead and chromium, *etc.* The toxic metal concentration as well as sodium, potassium, calcium gets magnified as the plant material is extracted with bore well water. Atomic absorption spectra, ICP spectra are useful to detect these metals. WHO standards are available to find out the permissible limits.

Yet another problem facing herbal formulation is deliberate mixing of allopathic drugs especially steroids or anti-inflammatory agents, *etc.*, to get effective remedy. Some anonymous journal expressed concern on adulterants in Asian patent medicines. The California Department of Health services, Food and Drugs branch initiated a study to screen "imported Asian medicines" to detect undeclared pharmaceutical and heavy metals by gas chromatography-mass spectrometry (GC-MS) and atomic absorption method. Eventually, a computer database will be created to educate consumers, herbal industry and medical community about the information on toxicity and potential dangers of the contaminated drugs. The data base also include microbiological studies to indicate, if the plant material is contaminated with harmful

E. coli and salmonella, *etc.* Such studies are lacking in India. Majority of commercial formulations contain these extracts mostly by aqueous extractions of multiherbal in the form of syrups, powders and tablets.

2. Discussion

It is clear from the mandate of AYUSH a close collaboration of AYUSH, with Central Govt. Laboratories (validation of formulations by identification of active principles by phytochemistry, quantification), *in vitro*, *in vivo* testing (Biological Institute), for phytodrugs *in silico* drug design, molecular drug design (Central Govt. Laboratories) clinical trials (Central Govt. Hospitals) and NGO's (Health camps) and popularization are necessary. Pharmacopoeia need to include HPLC analysis and chemical analysis and presence of elements.

Of particular concern is T.V and media advertisements on AYUSH based products (Ayurveda, Unani, Homeopathy) and in particular advertisements by Brand ambassadors ("I believe these medicines you believe it" is the slogan). These plant/mineral based traditional, proprietary medicines should come under the regulatory authority of AYUSH for License/approval.

3. Conclusion

AYUSH products need to be encouraged formulated as per their protocols need to be propagated at all costs. The reasons are many. We suggest AYUSH formulations need to be encouraged and promoted for various reasons. However, it is the duty of all branches of scientists and AYUSH to put these medicines on more "scientific and evidence based medicines." The antioxidant activity of these AYUSH medicines needs to be examined since they are beneficial to cure or prevent the disease. This particularly relevant because AYUSH became a full-fledged ministry in May 2014 with 1200 crores rupees budget for year. For the thousand of years that Ayurveda has been practiced but nobody asked for evidence based medicines. Now because these medicines are being exported, these questions are being asked as per the statement of Union Minister of AYUSH. There is need to coordination of AYUSH colleges with chemistry and pharmacology institutes of pharmacy and medical colleges with respect to teaching and practical's on isolation, processing of formulations, analytical estimations HPLC, HPTLC and profile by finger printing of the active principles, pharmacology, clinical trials and statistical analysis are necessary. Thus, there is an urgent need to undertake these interdisciplinary studies to make AYUSH a popular.

Among the various journals published on phytochemistry, pharmacology, ethnobotany, phytomedicines, *etc.*, '**Annals of Phytomedicine: An International Journal**' is vital platform for researchers and scholars including policy holders with its both online and print versions since a decade of its publication. The COVID special volumes are cited by other reputed journals, and the articles published in Annals of Phytomedicine are listed in WHO sites.

I am very confident that '**Annals of Phytomedicine: An International Journal**' with its committed scientific team of authors will continue to serve the societies in long future as well and I wish many more accomplishments and new achievements in its future endeavours.

Conflict of interest

The author declares no conflicts of interest relevant to this article.

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Biography

Dr. K. Nagaiah, Born 1966, Ph.D. Osmania University (OU), Hyderabad, 1995. Dr.Nagaiah, after obtaining his M.Sc. from Osmania University, pursued his doctoral work at Osmania University, Hyderabad and obtained his Ph.D. from Osmania University. Experience in the collection of plants by visiting forest and interaction with tribals to tribal medicine while working at Osmania University. After postdoctoral work on Organometal catalyst area with Prof. Phil Ho Lee, KISTEP Foundation Fellowship (Visiting Scientist) for Chemical Sciences 2001 for Post-doctoral studies in Kangwon National University, Chunchon, Republic of Korea. Experienced executive with multi-faceted skills, proven track record of innovation and exceeding organizational goals; Thirty-two years research leadership/accomplished project management/completion of several projects like Natural Products Isolation of biomarkers for Herbal drugs, Development including Process Chemistry, Industrial Chemistry and Good Laboratory Practices and can assist scientists, "young and old, shy and bold". My philosophy is that "it is better to be a guide by the side than a sage on the stage" and that it is better to "teach what you know, learn what you do not know". I encourage scholars to design, develop and carry research projects to fruition. He has developed synthetic strategies for several natural products and responsible for the structure determination of many of them. Natural Product Isolation, Total synthesis of complex Biological Active Natural Products and New synthetic methodology using metal catalyst. At present, he is serving as a Chief Scientist and Head, Centre for Natural Products and Traditional Knowledge and is actively engaged on the isolation of new chemical biomarkers for popular single plants drugs of AYUSH. Discovery and Development of New Chemical Entities, innovative synthetic routes/processes/formulations; I successfully carried out several industrial projects

with International companies like Smith Kline Beecham, UK (Solid Phase Organic Chemistry); ArQule, USA; Dupont, USA (New Chemical Entities); and Evolva, Singapore (New Chemical Entities) and Indigenous companies like Cipla (Dirthromycin), Sai Life Sciences (Solid Phase Organic Chemistry), Sami Labs Ltd (Solonasol), and Dolphin Laboratories (Nutraceutical). He also developed procedures for isolation of marker compounds for Nisarg Bio. Sci. Pvt. Ltd., Hyderabad, *etc.*, all these projects transfer by drug companies which also involve multistep organic synthesis. He has 18 Ph.D.s completed, 125 research publications and 3 national Patents to his credit, while, Ph.D. awarded 13 students, 4 students are working with him for Ph.D. He is recipient of several awards such Associate fellow of AP Akademi of Sci. (2002), He is a Fellow of AP Akademi of Sci. (2010). Ethical committee member, CRIUM, Hyderabad. Life Member of Catalysis Societies of India, 1992. Life Member of The Federation of Asian Biotech Associations (FABA), India. Life Member of India Science Congress Association. Life Member of Chemical Research Society of India. IISC, Bangalore. Life Member of Analytical Society of India, Hyderabad Chapter. He is a recognized research guide for Osmania University (Hyderabad), Kakatiya University (Warangal), Acharya Nagarjuna University (Guntur), JNTUH (Hyderabad) and Sri Krishnadevaraya University (Anantapur). Palamuru University (Mahabubnagar). BOS member at Vignana Bharathi Institute of Technology established by Swami Vivekananda Education Trust in 2004 to serve as Board of studies in Chemistry. Member Single Drug, Sub-Committee to Unani Pharmacopoeia Committee, CCRUM and Task Force Committee in CCRUM, Ministry of AYUSH, Govt. of India, New Delhi. Member of Board of Governors, NIPER, Kolkata (2022-25).