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Process, product and technologies for medicinal and aromatic plants

This article describes the Standard Operating Protocol (SOP) developments for 22 medicinal and aromatic plants. Also, some of the products and technology developed which could find societal benefits have been discussed here.

PRIMARY and secondary processing techniques play very important role in the quality and efficacy of the products prepared from raw material. Adoption of good agricultural practices, optimum harvesting time, drying techniques, storage conditions are required in order to ensure the best quality of raw materials. Good practices during bulk handling from field to storage and sale reduces the chances of contamination of the raw materials. Elimination of sources of contamination is also imperative for tackling the issue of microbial overload in herbal products.

Both conventional as well as advanced analytical techniques are widely being used for Standard Operating Protocol (SOP) development for high throughput fingerprinting of bioactive compounds for monitoring of quality of raw materials, extracts, enriched fractions as well as finished products. Isolation of bioactive phytochemicals and their characterization are important aspects of drug discovery and validation of pharmacological properties and traditional medicine. *In vitro* and *in vivo* studies establish the efficacy of extract, compounds and formulations or finished product. Chemometrics based studies have emerged as alternative to *in vitro* and *in vivo* studies for establishment of mode of action as well as other target specific behaviour of molecules.

The steps of post harvesting (both primary and secondary) for *Withania somnifera* (Ashwagandha) are depicted in Fig. 1.

SOP development is continuous research activity at ICAR-Directorate of Medicinal and Aromatic Plants Research, Anand. The SOP developed are summarized in Table 1.

Product development

Product development activities have been carried out in collaborative mode with research institutions under various externally funded projects (NASF, ICAR, DBT, NMPB) and in-house projects. Brief description of the some of the novel products developed are described below.

Novel, safe and stable anti-tick natural phyto-pharmaceutical formulation for the management of acaricide-resistant ticks infesting livestock and pet animals: Two flowable, stable, safe and potent anti-tick phyto-pharmaceutical formulations were developed. The lead compounds in the formulations are coumarin, precocene-I, β-caryophyllene oxide, α-humulene and β-caryophyllene. The developed formulations were subjected to acute and sub-acute oral and dermal toxicity as per OECD guidelines (425, 434, 407, 410) and found safe for animal and users.

The field trials were conducted as per the WAAVP guidelines. After establishing the *in vivo* efficacy, the field trials of the formulations were conducted in Maharashtra, Uttarakhand and Uttar Pradesh in organized and unorganized farms. The trials were conducted on 298 cattle. The post-application observations were collected after 7 days of application. The formulations were 80-85% effective against different stages of ticks infestations on animals (Fig. 2). As multiple principles are working in synergy in the developed formulations, the chance of development of resistance is comparatively low in comparison to chemical acaricides.

Standardized extract for growth inhibition of triple-negative breast cancer (TNBC): Extracts (G1 and G18) and purified phytochemicals (GI-PC

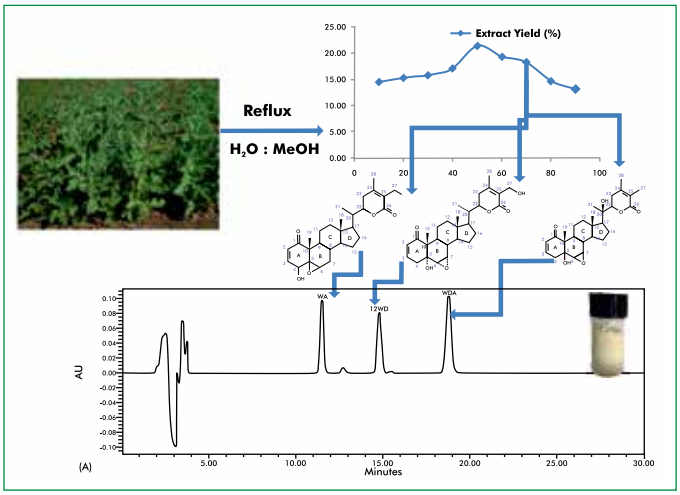

Fig. 1. Schematic diagram of processing steps for *Withania somnifera* (Ashwagandha)

Table 1. HPLC/UHPLC based developed SOP for medicinal and aromatic plants

Name of plant	Parameter for optimization of extraction process	Selected marker (bioactive compound)	Chromatographic/ spectroscopic method used
1. *Ageratum conyzoides* (**Jungli pudina**)	Chromenes, coumarins, sesquiterpenes	Coumarin, precocene-I, β-caryophyllene oxide, α-humulene, and β-caryophyllene	HPLC-PDA
2. *Andrographis paniculata* (**Kalmegh**)	Andrographolides	Andrographolide, neo-andrographolide and andrograpanin	HPLC-PDA
3. *Asparagus racemosus/ adscendens* (**Shatavari**)	Saponins	Shatavarin IV	TLC and HPLC-ELSD UV-Visible spectrophotometry (for total saponins)
4. *Cassia* species (**Senna**)	Sennosides	Sennoside A, B	HPLC-PDA
5. *Cassia tora* (**Chakwad**)	Anthraquinone derivatives	Rhein, emodin, chrysophanol, physcion	HPLC-PDA/MS
6. *Chlorophytum borivilianum* (**Safed musli**)	Saponins	-	TLC and HPLC-ELSD UV-Visible spectrophotometry (for total saponins)
7. *Desmodium* species (**Salparni**)	Total phenolics	Gangetinin and phenolic acids (gallic, caffeic, chlorogenic, sinapic)	HPLC-PDA/MS
8. *Eclipta alba* (**Bhringraj**)	Wedelolactone	Wedelolactone	HPLC-PDA
9. *Enicostemma axillare* (**Mamejo**)	Swertiamarin	Swertiamarin	HPLC-PDA
10. *Garcinia* species (**Kokam**)	Polyisoprenylated benzophenone and xanthone	Xanthochymol, isoxanthochymol, Mangostin (α, β and γ)	UHPLC-PDA
11. *Gymnema sylvestre* (**Gurmar**)	Gymnemagenin / Gymnemic acid	Gymnemagenin	HPLC-PDA
12. *Leptadenia reticulata* (**Dodi**)	Flavonoids	Apigenin, diosmetin, Luteolin	HPLC-PDA/MS
13. *Lepidum* sativum (**Asalio**)	Phenolic acids and glucosinolates	Gallic, caffeic, chlorogenic, sinapic Sinigrin	HPLC-PDA
14. *Mucuna pruriens* (**Kauncha**)	L-DOPA	L-DOPA	HPLC-PDA
15. *Phyllanthus* species (**Bhui Amla**)	Lignans and tannins	Phyllanthin, hypophyllanthin, niranthin, nirtetralin gallic acid, corilagin, chebulagic acid, ellagic acid and chebulinic acid	HPLC-PDA/MS
16. *Piper* species (*P. longum*)	Piperine and phenolics	Piperine	HPLC-PDA
17. *Plumbago zeylanica* (**Chitrak**)	Plumbagin	Plumbagin	HPLC-PDA
18. *Saraca asoca* (**Asoca**)	Catechins	Catechin, gallic acid, procyanidin-B2, (-)-epigallocatechin gallate, (-)-epicatechin gallate	UHPLC-PDA
19. *Taverniera cuneifolia* (**Jethimadh**)	Glycyrrhizic acid, glabridin	Glycyrrhizic acid, glabridin	HPLC-PDA/MS
20. *Terminalia* species (*T. arjuna, T. bellerica, T. chebula, T. catappa*) (**Arjuna)**	Phenolic/tannins	Gallic acid, corilagin, chebulagic acid, ellagic acid and chebulinic acid	HPLC-PDA
21. *Withania somnifera* (**Ashwagandha**)	Withanolides	withaferin A, 12-deoxywithastromonolide and withanolide A	HPLC-PDA
22. *Vitex* species (*V. negundo* and *V. trifolia*)	p-hydroxybenzoic acid, negundoside and agnuside	p-hydroxybenzoic acid, negundoside and agnuside	HPLC-PDA

Infestation before application Efficacy after 7 days of application

Fig. 2. Field evaluation of anti-tick natural phyto-pharmaceutical formulation

and GM-PC) were evaluated for anti-TNBC properties on subcutaneous tumours of TNBC (MDA-MB-231) established in immunodeficient NOD-SCID mice. When G18 phytoextract was fed to NOD-SCID mice via oral route, it caused significant tumor growth inhibition (58%; p=0.031) of the MDA-MB-231 xenograft tumours. These compounds exhibited no toxic effect on mice after treatment, based on 100% survival at the end of treatment and no change in animal total body weight. Underlying mechanism of anti-cancer activity of G18 phytoextract was evaluated *in vitro* by cell cycle analysis and changes in ultrastructure morphology by electron microscopy. G18 caused arrest of MDA-MB-231 cells in G0-G1 cell cycle phase (untreated G1 phase 53%; G18 40 µg/ml 86%) and induced apoptosis (untreated apoptosis 1.7%; G18 40 µg/ml 25%) in dose-dependent manner. At ultra structural level, G18 caused reduction in cell-cell communication, endoplasmic reticulum (ER) dilation, loss of integrity in mitochondrial cristae, nucleus and cell cytoplasm of MDA-MB-231 cells, leading to decreased cell proliferation.

As the MDA-MB-231 ($ER^-/PR^-/HER2^-$) cells are highly metastatic and more aggressive as compared with other for breast cancer clinical type such as MCF-7 ($ER^+/PR^+/HER2^-$), these extracts based natural formulation may serve as adjuvant for TNBC chemotherapy. Based on the results, it was concluded that G18 can be developed as potential drug candidate for triple-negative breast cancer therapeutics.

Extracts and purified phytochemicals from *Garcinia* as cost-effective therapeutics for management of colon cancer: Efficacy of extracts (G1, G18) and purified phytochemicals (GI-PC and GM-PC) were investigated *in vitro* and *in vivo* against Colo-205 human colorectal carcinoma on a pre-established subcutaneous tumor of Colo-205 in immunodeficient NOD-SCID mice for development of cost-effective therapeutics and chemoprevention agent. GI-PC and GM-PC caused significant growth inhibition (68% and 75% tumor growth reduction, respectively) of the subcutaneous xenograft tumors of Colo-205 in immunodeficient mice. These compounds exhibited no toxic effect on mice after treatment, based on 100% survival at the end of treatment and no change in animal total body weight. GI-PC and GM-PC could be developed as potential drug candidates for colon cancer therapeutics.

Microencapsulated herbal product with ovicidal and larvicidal properties against *Ancylostoma duodenale* (Hookworm): Despite considerable advances in chemotherapy and control, hookworms rank amongst the most widespread of soil-transmitted intestinal helminths especially in the tropics and sub-tropics. Human hookworms (*Ancylostoma duodenale*) are among the prominent soil transmitted helminths (STH) with a global burden of nearly 740-1300 million affected cases. The strategy adopted for global control of helminths relies predominantly on mass drug administration (MDA) of anthelmintic drugs to the targeted population. However, similar strategies in the veterinary population have resulted in considerable rates of anthelmintic resistance. About 25% of the global burden of STH infection is contributed alone by India with high prevalence of hookworm infection.

Ovicidal and larvicidal properties of microencapsulated herbal products (APSD3 and APSD5) from an Indian medicinal plant were validated on field isolates of *A.duodenale*. APSD3 and APSD5 exhibited ovicidal and larvicidal properties which were comparable and better than the standard drug albendazole and therefore APSD3 and APSD5 could be explored as potent therapeutic agent for hookworm infection in human.

Fruit juice of *Garcinia indica* Choisy. for modulating dyslipidemia and lipid metabolism: The prevalence of dyslipidemia leading to obesity is rapidly increasing; however, limited medications are presently available in the market. Obesity is a dyslipidemic disorder, wherein, derangement in lipid metabolism has been seen along with abnormal lipid levels, often associated with higher storage of lipid in adipocytes. It is interesting to note that dyslipidemia is associated with a cluster of diseases thereby, being a central player in development of metabolic syndrome. The manifestation of syndrome has decreased life expectancy and its quality.

G. indica fruit juice was standardised for the hydroxycitric acid content and its efficacy for modulating dyslipidemia and lipid metabolism were validated in cafeteria diet-fed rat model. Results showed that cafeteria diet fed animals exhibited increased body weight, increased food intake, decreased water intake, increased glucose intolerance and dyslipidemia. Treatment with *G. indica* fruit juice for 4 weeks, reduced the body weight, improved the metabolic parameters like glucose sensitivity, dyslipidemia, insulin and leptin levels and lipid metabolizing levels without causing toxicity. The results obtained were better than orlistat, which is a standard mode of chemotherapy for management of dyslipidemic obesity.

Standardized extracts from exhibiting potential antibacterial activity against methicillin-resistant *Staphylococcus aureus:* Antibacterial activity of 59 standardized extracts was screened against methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 33591) and methicillin-sensitive *Staphylococcus aureus* (MSSA, ATCC 25923). The antibiofilm formation effects of the extracts with significant antimicrobial activity were also studied. Extracts M10, M12, M24, M36, M45, M51

and M58 showed significant antimicrobial activity and lower MIC against the ATCC strains of *S. aureus* as compared to the standard antibiotics. These extracts also showed very low MIC against 5 clinical isolates of MRSA. Extract M24 showed anti-biofilm activity at all the concentrations starting from 0.25 g/ml. Extract M12 and M36 showed inhibition but at higher concentrations. Extract M10 and M45 showed no anti-biofilm activity at any concentrations. However, extract M51 and M58 showed anti-biofilm activity at lower concentrations but no inhibition at higher concentrations. These extracts could find wide pharmaceutical use as they were as efficacious as ciprofloxacin.

Technologies developed

Supercritical Fluid Extraction of Medicinal Plants: Supercritical fluid extraction (SFE) was carried out using pilot scale equipment with a 1-L extraction vessel (Fig. 3). Optimization of parameters such as automated back pressure, carbon dioxide flow, modifier composition, extraction time and the temperature of the extraction vessel were optimized using Super Chrom SFC suit v5.9 software for extraction.

Andrographis paniculata (*Kalmegh*): The solid–liquid extraction methods resulted in extraction of diterpenoids in the range of 9.76–66.95 and 3.55–33.42 mg/g for AP and neoandrographolide, respectively, however, for SFE, it was 131.82 mg/g for AP and 21.75 mg/g for neoandrographolide, respectively

Cassia angustifolia (*Senna*): Senna is a high value crop and its whole plant is medicinally important mainly for its cathartic property. The active principles of the widely used senna extract are the anthraquinones, in addition to the senna specific dianthrones, called sennosides.

Extract yield under optimized parameters of SFE (SC-CO_2 flow rate (g/min) 30; Modifier (g/min) 4, Automated back pressure, 100 bar; Temperature 60° C and Extraction time 2 hour) was 100.9 mg/g and sennoside content (mg/g + SD) (SA= 1.48 + 0.18). Although the sennoside content was lower in SFE extract, however, some other major peaks were also recorded in this extract which needs identification.

Withania somnifera (Ashwagandha): W. somnifera (commonly known as Ashwagandha), is being used in Indian System of Medicine for over 3,000 years in various forms (decoction, infusion, ointment, powder and syrup) and also in different parts of the world. Withanolides, polyoxygenated C28 steroidal lactones are the major chemical constituents of *Withania* species and are present in almost all parts of *W. somnifera*. Extract yield from SFE was 2.46%. TPC in the extract prepared by SFE was 2.92%. WA, 12WD, and WDA concentrations in SFE samples were 0.005, 0.231, and 1.101%, respectively. TPC in SFE extract was comparable with water-methanol (70:30) TPC (2.92%). However, total withanolide content was about 20.51 times higher in SFE extract.

Phyllanthus amarus (Bhui amla): In recent years, interest in the plants of the genus *Phyllanthus* has increased considerably, especially regarding their therapeutic potential for the management of many diseases. *P. amarus* is one of the most studied plants due to its hepatoprotective properties. Process was optimized for preparation of extracts enriched with lignans (Ph+HPh+NT+NR), corilagin and ellagic acid.

Garcinia species (Kokam): Garcinia species were investigated worldwide for a large variety of biologically active secondary metabolites. In India, 43 species and 5 varieties of *Garcini*a are reported. *Garcinia* species are a rich repository of diverse secondary metabolites such as xanthones, benzophenones, biflavonoids, flavonoids, biphenyls, acyl phloroglucinols, etc. More than 17 *Garcinia* species have been investigated by us for the search of anticancer compounds (polyisoprenylated benzophenones and xanthones). In continuation with the work, SFE process has been optimized for preparation of extract with high content of PIB and xanthones.

Fig. 3. Super critical extraction facility at ICAR-DMAPR, Anand

Technology for high content of catechin extracts from a renewable source: Catechins are well known flavonoids used for the symptomatic treatment of several gastrointestinal, respiratory, and vascular diseases. A process was developed for preparation of the catechins rich extract from the leaves of *S. asoca* with catechin content 33.33±0.06 mg/g. Furthermore, this renewable source could find application as a substitute for bark of *S. asoca* (*catechin content 10.23±0.06 mg/g*) in different herbal formulations such as Ashokarishta, Ashokaghrita, etc (Fig. 4). This would prevent the destructive harvesting of the *S. asoca* plant, thereby, promoting its conservation.

Technology for green synthesis of gold nanoparticles mediated by *Garcinia* fruits: Green synthesis of gold nanoparticles (AuNPs) using medicinal plant extract is an emerging area due to their applicability in nanomedicine.

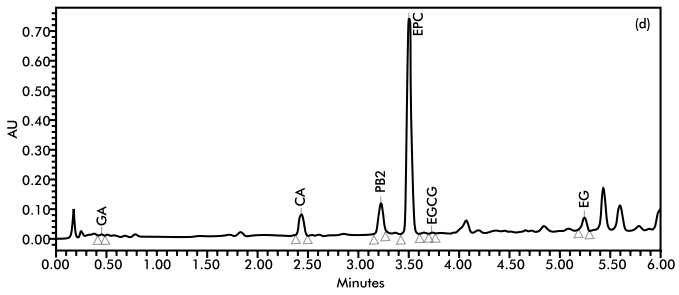

Fig. 4. UPLC-PDA chromatogram of *S. asoca* leaves extract

Aqueous extracts prepared from fruit-pericarps of two *Garcinia* species, *G. indica* (GI) and *G. cambogia* (GC) fruits which are important medicinally and commercially were utilized for the synthesis of AuNPs. Various analytical techniques were utilized to characterize the synthesized AuNPs. AuNPs exhibited considerable cytotoxicity with IC_{50} values 34.55 µg/ml (GI) and 35.69 µg/ml (GC) against the MCF-7 cancer (human breast cell line). Furthermore, synthesized AuNPs also demonstrated significant antioxidant and antibacterial properties comparable to the standards used.

Technology for isolation of high value compounds: Rapid isolation methods have been developed for high value compounds such as xanthochymol, isoxanthoichymol, -mangostin, andrographolides, wedelolactone and negundoside. These compounds could find demand as reference standards for research as well as industrial purposes.

SUMMARY

Standard Operating Protocol (SOP) developed could find application by researchers and other stakeholders of the medicinal and aromatic plants for quality control of the raw and finished materials as well as for exploring the other sources of selected marker compounds from the nature. Products and technology developed may find application in societal benefit and also creating more avenues in this emerging area of importance.

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Book Review

ROSES IN THE FIRE OF SPRING

The book '*Roses in the Fire of Spring*' is a treatise on the life's journey with roses for the legendry duo M S (Viru) and Girija Viraraghavan.

In this book, the winners of the 'Great Rosarian of the World' award of the important Huntington Gardens, California, have shared rare insights into the domain of rose improvement. Awarded the 'World Rose Award' by the World Federation of Rose Societies (WFRS), Viru is a respected name in the global rose world today. Both Viru & Girija have won many honours for their work. They have also worked extensively on rhododendrons and many other important flowers.

In the last more than four decades, Viraraghavans have added more than 100 new roses to the wealth of Indian roses. Not all are based on the two wild species *(Rosa clinophylla* and *Rosa gigantean*), but have used the best performing roses in different parts of the country as parents. Viru is also credited with developing, perhaps the first HT type 'hand painted' rose – *Priyatama*, named after Girija. Many of his roses are performing well in many countries abroad, in Asia, Europe, USA, Australia, South Africa, New Zealand, etc. A few nurseries in Europe and elsewhere have started stocking roses bred by Viraraghavans for their customers, perhaps the only rose breeder of the region to have the privilege.

The book details their travels to many famous rose gardens in the world and their learning from discussions with many eminent rosarians of the world at the many International rose meetings they have been invited to address. They have spoken and written extensively for the Indian rosarians at the many Indian Rose Conventions and in 'Indian Rose Annual'.

A special feature of Viraraghavans has been the naming of their varieties. The book gives detailed descriptions of all their varieties, with the characteristics and history behind many of them. For many varieties, popular abroad, they have two names, one for the rosarians abroad who find it difficult to correctly pronounce some of the tongue twisting Indian names. They have named many roses recognising the contributions of several pioneer Indian rose breeders like B.S. Bhatcharji or Swami Vinayananda or acknowledging the support of Dr N.C. Sen and Narender Singh, who got the *R. clinophylla* plants for them or Sir George Watt and Sir Henry Collett, who discovered the virtues of *R. gigentea* in 18th century.

The book is a reservoir of rose knowledge gathered by Viraraghavans over nearly last five decades and besides giving insights into their thought process in rose breeding, describes the beauty of several roses found in gardens abroad.

Dr Narendra Dadlani

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