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Polyherbal formulations: Assessment of metal toxicity, pesticide and microbial contamination

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Abstract : Medicinal plants are widely explored worldwide for their medicinal benefits. They are intrinsic components of traditional system of medicines and complementary alternative medicine (CAM). However, heavy metals, microbial and pesticide contamination in soils has turn out to be one of the major challenges faced by herbal medicine makers. Disparity in the levels of heavy metal, micro organisms and pesticides add on contamination and adverse effects in herbal preparations. Possibility of contamination and adulterants is more in polyherbal formulations as they are derived from diverse plant herbs. Our study was aimed at determining heavy metal concentration and pesticide residue in the selected traditional herb components that make up'*Laksha Guggulu'* formulation from different places of Maharashtra, India. total of 12 samples from six plants (two samples for each plant) were analyzed for their heavy metals and pesticide contents by plasma emission spectrophotometer and gas chromatography techniques. In these samples, Mercury (Hg), Lead (Pb), Cadmium (Cd), Chromium (Cr) and Nickel (Ni) were present in all samples however, below the permissible limits. In few samples, Pb and Cd contents were beyond the WHO permissible limits. Apart from these, isomers of α-HCH and γ-HCH pesticides residue were present in almost all the samples. Yet, other pesticides such as β-HCH, DDT and DDE were not detected in these samples. δ-HCH was found merely in three samples. Thus, this study would serve as a prototype profiling layout for weighing the safety and contamination of polyherbal formulations prior to their safe use.
Keywords : Polyherbal formulation, Heavy metals, Organochlorine pesticides, Microbial contamination.

Introduction

Medicinal plants are starting material for any herbal formulations.Herbal drug is a principal constituent in traditional medicine. Herbs are usually considered as safe since they belong to natural sources as compared to allopathic medicines. Medicinal plants are consumed worldwide forthe treatment of several diseases due to their

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therapeutic potential and are importantraw materials in the production of phyto-pharmaceuticals for the pharmaceutical industry.

In recent decades, the use of phyto-pharmaceuticals and herbal medicines has increased worldwide,for several reasons, among them, that side-effectare often lower than those presented when syntheticdrugs are employed, as well as due to the higher costs of many conventional pharmaceutical formulations. As with other vegetation, medicinal plants are composed of many constituents andpresent great variability due to different growth, harvest, drying and storage conditions[1-3].

Medicinal herbs are moving from fringe to mainstream use with more number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Considering the adverse effects of synthetic drugs, the world population is looking for natural remedies which are safe and effective. Lead(Pb), cadmium (Cd), chromium (Cr), nickel (Ni), arsenic (As) and mercury (Hg) are the most common toxic metals that have become a matter of concern due to the reports of their contamination in various herbal preparations and herbal ingredients[4-6].

The evaluation of toxic heavy metals,microbial content and pesticide residual levels in finished Ayurvedic product has become essential for formulations being used by a large andgrowing population.Hence, the aim of present research is to check the level of toxic contaminants such as heavy metals, viz. As, Hg, Pb, Cd, Cr and Ni, organochlorine pesticides residues, viz. isoforms of HCH, DDT, DDE and micro organisms in different samples of '*LakshaGuggulu*'[7-8].

Materials and Methods

Plant material

The plants *Lacciferalacca, Withania somnifera, Terminalia arjuna, Cissus quadrangularis, Commiphora wightii* and *Sida veronicaefolia* were purchased from Sanjeevani Ayurveda, Pune, Ayurvedic Rasashala, Kolhapur Yucca Enterprises, Wadala(E) Mumbaiand were authenticated by Agarkar Research Institute, Pune. The drugs were sun dried, coarsely powdered and stored in airtight containers. All chemicals and reagents used were of LR grade.

Instruments

Varian Gas Chromatography/Mass Spectrophotometer and Inductively Coupled Plasma Emission Spectrophotometer (ICP 8440 Plasma,Labtam) were employed for analysis of pesticide residues and heavy metals.

Preparation of samples[9-18]

Total 12 samples belonging to 6 plant species used as 'Laksha Guggulu' was taken for the study (Table 1) and purchased from 3 different sources in the local markets of Pune, Mumbai and Kolhapur, India. Fresh samples were air-dried at room temperature. All samples were powdered, sieved, and stored in plastic bags. Heavy metals, specific microbial contaminant analysis and organochlorine pesticides residues of different metabolites of DDT, DDE, isomers of HCH (α, β, δ,γ) were estimated according to reference procedure and Ayurvedic Pharmacopoeia.

Table 1: Plant species and their places of collection

Common Name	Plant species	Place of collection
Laksha	*Lacciferalacca*	Pune and Kolhapur
Ashwagandha	*Withaniasomnifera*	Pune and Mumbai
Arjuna	*Terminaliaarjuna*	Pune and Kolhapur
Asthisamharaka	*Cissusquadrangularis*	Pune and Kolhapur
Guggul	*Commiphorawightii*	Pune and Mumbai
Nagabala	*Sidaveronicaefolia*	Pune and Kolhapur

Heavy metal analysis

1 g powder of each sample was used. All of the chemicals used were of analytical grade. Samples were digested in nitric acid and Perchloric acid (HNO_3: $HClO_4$, 6:1), using a wet digestion method, by heating slowly on a hot plate until a white residue was obtained under the fume hood chamber. The residue was dissolved in 0.1 N Nitric acid and diluted to a volume of 10 mL. The digested samples were analyzed on an inductively coupled plasma emission spectrophotometer. The standard reference samples of As, Hg, Pb, Cd, Cr and Ni were used to establish a calibration curve and to validate the metal content of each analytical sample.

Microbial contamination study

For microbial contamination study, total 12 samples belonging to 6 plant species were inoculated in Mac Conkey broth medium followed by 36 to 38 ^{0}C incubation for 48 hours. Various staining methods were employed for identification of microbial contaminants if any. Primary test was performed for acid and gas production in the test tube using MacConkey broth medium post incubation. Secondary test was carried out for indole using Peptone water medium incubation at 43.5to 44.5^{0}C for 24 hours. After incubation Kovac's reagent was added for checking presence of indole.

Additional tests were carried in autoclaved Fluid Soyabean-Casein digest medium after it was incubated at 35-37^{0}C for 48 hours. After incubation, take sterilized wire loop. Cool the loop by touching it on the edge of the sterile Cetrimide agar plate. Dip the loop laden with test samples into Fluid Soyabean-Casein digest medium. Lift the lid of the plate just enough to insert the loop. Drag the loop over the surface of the top one-third of the plate back and forth in a 'zig-zag' formation. Streak a portion of the medium on the surface of Cetrimide agar medium, each plated on petri dishes and cover it. The loop if contaminated with bacteria would spread them over the surface of the agar.Further, sterilize the loop, turn the plate 90 degrees and drag the loop through the area you have just streaked two to three times and continue to drag the loop in a 'zig-zag' formation in the remaining half of the plate without touching that area again. Sterilize the loop in the flame. Turn the plate 90 degrees. Repeat the procedure. Drag the loop two to three times through the area you just streaked and streak in the remaining area of the plate (zig-zag formation). One has to be cautious to avoid the areas previously streaked. Incubate at 35-37^{0}C for 18 to 24 hours. If you streaked correctly, you will see isolated contaminant colonies in the third sector. The heaviest growth will be in the first sector. There will be less growth and some isolated colonies in the second sector. The third area would depict least growth with isolated contaminant colonies.

Organochlorine pesticides residues:

To determine the organochlorine pesticides residues of different metabolites of DDT, DDE, isomers of HCH (α, β, δ, γ), approximately 2g herbal powder (sieved through a No. 40 mesh sieve) was weighed and added into a 100 mL centrifuge tube for analysis. 8 mL water and 10 mL acetonitrile were added and vortexed for 1 min. After this, 1.0 g NaCl and 4.0 g $MgSO_4$ were added, andvortexing was repeated for 1 min. The extract was then centrifuged (4000 rpm) for 5 min. A 1.0 mL supernatant (acetonitrile phase) was then transferred to a single use centrifuge tube which contains following sorbents: 300 mg primary secondary amine, 50 mg Graphitic Carbon Black, 50 mg anhydrous $MgSO_4$. Postvortex-mixing for 1 min, the mixtures were centrifuged at 12000 rpm for 5 min and the cleaned extract was transferred into a vial for subsequent analysis. Aliquots of above concentrate were injected into precalibrated GC/MS. Purified nitrogen gas was used as carrier gas. Periodically procedural blanks were used to check cross contamination.

Result and Discussion

Plant species viz. *Laccifer lacca, Withania somnifera, Terminalia arjuna, Cissus quadrangularis, Commiphora wightii* and *Sida veronicaefolia* were collected from different places of Maharashtra. Heavy metals levels (Table 2) were estimated in the six main ingredients of 'Laksha Guggulu' and evaluated with respect to permissible limit as prescribed by the WHO. Heavy metals viz.As, Pb, Cd, Ni, Hg and Cr were assessed in these samples and observationswere as indicated in Table 2.'As' was not detected in most of the samples whereas 'Pb' was detected in all the samples; in two samples, 'Pb' content was even beyond the WHO permissible limit i.e. 10 mg/kg. Maximum 'Pb' was present in *Terminalia arjuna* and *Commiphora wightii* i.e. 13.83 mg/kg and 16.66 mg/kg, respectively. 'Cd' was well below the WHO permissible limit (0.3 mg/kg) in

almost all the samples except two viz *Terminalia arjuna* and *Sida veronicaefolia* (0.389 mg/kg and 0.39 mg/kg). Minimum 'Ni' was found in *Withania somnifera* and *Commiphora wightii* and maximum in *Terminalia arjuna*. However, the significant variations were observed in 'Hg' and 'Cr'contentfor all the samples; it ranges between minimum 1.121 mg/kg for 'Hg' and 1.42 mg/kg for 'Cr' in *Lacciferalacca* and maximum 2.462 mg/kg in *Withania somnifera* for 'Hg' and 8.745 mg/kg in *Sida veronicaefolia* for 'Cr'.

Table 2: Heavy metals concentration (mg/kg) in selected medicinal plants samples

Plant name	Place	Cadmium (Cd)	Arsenic (As)	Lead (Pb)	Nickel (Ni)	Mercury (Hg)	Chromium (Cr)
Laccifera lacca	Pune	BDL	BDL	2.766	BDL	1.121	1.42
	Kolhapur	BDL	BDL	2.481	BDL	1.240	1.51
Withania somnifera	Pune	BDL	BDL	5.522	3.71	2.462	BDL
	Kolhapur	0.421	BDL	5.643	BDL	2.102	BDL
Terminalia arjuna	Pune	0.3889	BDL	13.83	7.49	1.570	2.85
	Mumbai	0.2867	BDL	12.41	6.94	1.342	2.81
Cissus quadrangularis	Pune	0.0975	BDL	6.94	6.24	1.8	2.86
	Kolhapur	0.0843	BDL	5.96	BDL	1.67	2.76
Commiphora wightii	Pune	0.1951	BDL	16.66	3.73	1.576	4.318
	Mumbai	0.1856	BDL	12.11	3.01	1.25	BDL
Sida veronicaefolia	Pune	0.39	BDL	6.93	7.52	1.125	8.745
	Kolhapur	0.35	BDL	6.21	BDL	1.012	BDL

**Values are average of three replicates; SD not more than ±5% in each case.*
**BDL: below detection limit*

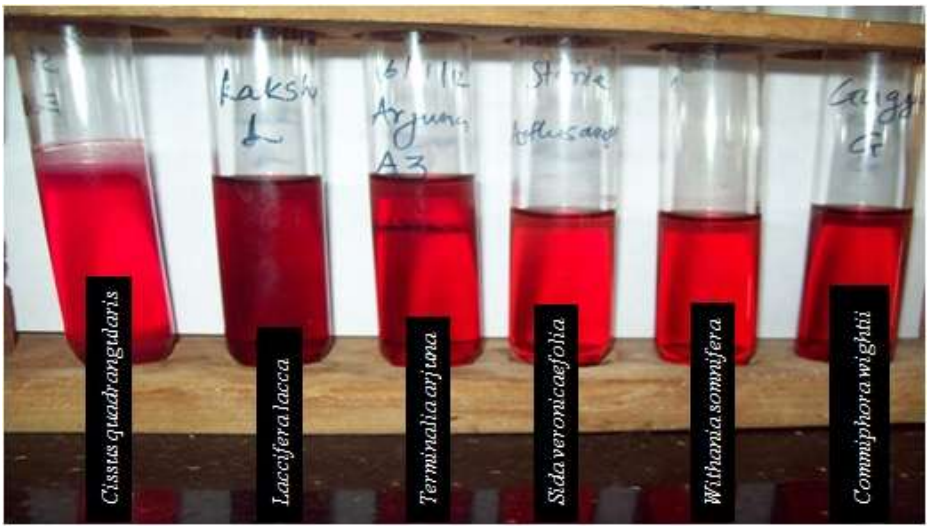

Fig. 2:Absence of acid, gas and Indole formation for all the samples in MacConkey's broth

Laksha Guggulu' showed absence of acid as well as gas production during microbial evaluation. Also, negative Indole test in the primary and secondary screening test indicated absence of *Escherichia coli* as shown in the Fig.2. The results were as expected and presented in Table 3.

Table 3: *Escherichia coli* detection by acid, gas and indole formation in primary and secondary screening of polyherbal samples.

Samples	Acid production	Gas formation	Indole formation	Inference
Commiphorawi ghtii	-	-	-	Absence of typical coliforms like *E. Coli*
Terminaliaarju na	-	-	-	Absence of typical coliforms like *E. Coli*
Laccifera lacca	-	-	-	Absence of typical coliforms like *E. Coli*
Sidaveronicaef olia	-	-	-	Absence of typical coliforms like *E. Coli*
Withaniasomni fera	-	-	-	Absence of typical coliforms like *E. Coli*
Cissusquadran gularis	-	-	-	Absence of typical coliforms like *E. Coli*

Another microbial test performed in Fluid Soyabean-Casein digest medium illustrated absence of green colored colonies post streaking under ultra-violet light (Fig. 3). Thus, the tested samplesexhibited absence of *Pseudomonas aeruginosa*.

The presence of total hexachlorocyclohexane (HCH) and its isomers in all the samples were evaluated as tabulated in Table 4. It is evident from the data that γ isomer of HCH was more prominent in comparison to all other isomers viz. α, β and δ. β-HCH was not detected in any of the sample while δ-HCH was detected only in five samples. Kolhapur sample of *Laccifer lacca* had maximum δ-HCH i.e. 0.0065 mg/kg; other five samples which showed the presence of δ-HCH were *Withania somnifera*(Kolhapur), *Terminalia arjuna*(Kolhapur and Pune),*Cissus quadrangularis*(Kolhapur), *Commiphora wightii*(Kolhapur) and *Sida veronicaefolia*(Pune). In *Withania somnifera*, *Cissus quadrangularis*, *Commiphora wightii* and *Sida veronicaefolia* (Pune), α-HCH was below detection limit. DDT and DDE were not detected in any of the samples.

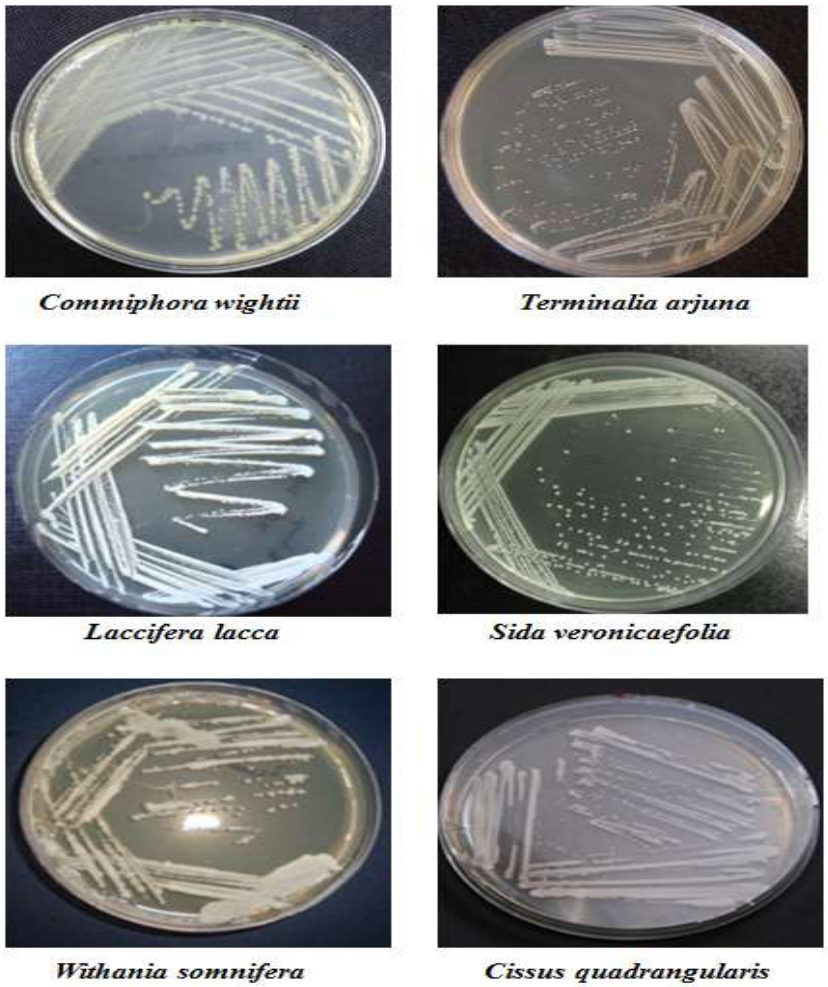

Fig.3: Microbial screening test of polyherbal samples in Fluid Soyabean-Casein digest medium for *Pseudomonas aeruginosa*.

Only two samples showed the presence of DDT and DDE. In *Terminalia arjuna*(Mumbai), DDE (0.0102 mg/kg) and *Withania somnifera* (Kolhapur), pp-DDT (0.0005 mg/kg) and pp-DDE (0.0086 mg/kg) were detected. However, presence of α-HCH and γ-HCH, the main constituents of commercial HCH was detected in 95% samples. Out of 12 samples analyzed in this study, it is clear that only 5% contain residue of DDT and its metabolites. This indicated that residual build up is more of HCH in plant samples analyzed as compare to DDT.

Table 4: HCH residues (mg/kg) in selected medicinal plants samples

Plant name	Place	α-HCH	β-HCH	γ-HCH	δ-HCH	Total HCH
Laccifera lacca	Pune	0.0001	BDL	0.0039	BDL	BDL
	Kolhapur	0.0025	BDL	0.0030	0.0065	BDL
Withania somnifera	Pune	BDL	BDL	BDL	0.0031	BDL
	Kolhapur	BDL	BDL	BDL	BDL	BDL
Terminalia arjuna	Pune	BDL	BDL	BDL	0.0021	BDL
	Mumbai	BDL	BDL	BDL	0.0014	BDL
Cissus quadrangularis	Pune	0.0039	BDL	BDL	0.0005	0.0017
	Kolhapur	0.0012	BDL	0.0015	BDL	BDL
Commiphora wightii	Pune	BDL	BDL	0.0010	BDL	BDL
	Mumbai	BDL	BDL	BDL	BDL	BDL
Sida veronicaefolia	Pune	BDL	BDL	BDL	BDL	BDL
	Kolhapur	BDL	BDL	BDL	BDL	BDL

Conclusion

From this study, it can be seen that some metals such as Pb, Cr and Ni, pesticide residues such as γ-HCH and δ-HCH are present in herbal drugs at levels higher than the permissible limit which may cause serious health problems. Therefore, care must be taken to avoid such toxic materials from herbal preparation, especially in large doses. It must be mandatoryto evaluate the heavy metals, pesticide residues and microbial contamination in every batch of polyherbal preparations and their individual ingredients prior to formulation. This will aid in usersafety and improve the export prospective of herbal drugs.

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