Section A -Research paper
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STUDY OF HEAVY METAL, PESTICIDE RESIDUE AND ELEMENT ANALYSIS OF *AMRTOTTARA KWATHA CHURNA*: AN AYURVEDIC FORMULATION''

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ABSTRACT

For the treatment of several diseases medicinal plants are consumed worldwide due to their therapeutic potential. Medicinal plants are important raw materials in the production ofphyto-pharmaceuticals for the pharmaceutical industry. Medicinal plants may be easily contamination of medicinal plants m by absorbing heavy metals from soil, water and air. Due to their cumulative behavior and toxicity, however, they have a potential hazardous effect not only on crop plants but also on human health. The residues of pesticides including their metabolites and/or degradation products will remain in plants, or in the soil that become a notable source of contamination for herbal medicines. However, heavy metals, microbial and pesticide contamination in soils has turn out to be one of the major challenges faced by herbal medicine makers. Our study was aimed at determining heavy metal concentration and pesticide residue in the selected medicinal plants that make up different formulations of *Amrtottara Kwatha churna*. In all three formulations of *Amrtottara Kwatha churna* heavy metals like Arsenic (Ar), Cadmium (Cd), Lead (Pb) and Mercury (Hg) were present within the permissible limits. Elements like carbon, hydrogen and nitrogen contents were present in all the formulations.

Keywords: *Amrtottara kwatha*, Churna, Ayurvedic Formulation, Heavy metal.

INTRODUCTION

Medicinal plants are starting material for any herbal formulations. In traditional medicine herbal drug is a principal constituent. Herbs are belonging to natural sources that's why considered as safe as compared to allopathic medicines. Medicinal plants have high therapeutic potential so they are consumed worldwide for the treatment of several diseases. Medicinal plants are important raw materials in the production of phyto-pharmaceuticals for the pharmaceutical industry [1]. Medicinal plants may be easily contaminated by absorbing heavy metals from soil, water and air. Usually soil is subjected to contamination through atmospheric deposition of heavy metals from point sources including different industrial activities. Additional sources of these elements for plants are rainfall, atmospheric dusts and plant protection agents Toxic elements from wastewater may contaminate agricultural soils, water supplies and environment and hence human food chain. The residues of pesticides including their metabolites and/or degradation products will remain in plants, or in the soil that become a notable source of contamination for herbal medicines. Organochlorines are central nervous system stimulants that can cause tremors, hyperexcitability and seizures. Although these pesticides are generally less acutely (immediately) toxic than organophosphates or carbamates, since they persist in the environment and tend to accumulate in tissue as they pass up the food chain, they are extremely hazardous. Organochlorine pesticide residues and breakdown products are found in human breast milk worldwide, and also in soil and plant and animal tissue from the middle of the Pacific Ocean to the Arctic Circle **[2].** Heavy metals are those in their standard state and have a specific gravity of more than 5 g cm-3. Some of the heavy metals are essential in very low concentrations for the survival of all forms of life. Trace heavy metals are important in daily diets, because of their essential nutritious value and possible harmful effects. Heavy metals such as iron, chromium, copper, zinc, cobalt, manganese and nickel are essential metals since they play an important role in biological systems; whereas mercury, lead and cadmium are non- essential metals which can be toxic even in trace amounts. After collection and transformation of herbs into dosage form, the heavy metals confined in plants finally enter the human body and may disturb the normal functions of central nervous system, liver, lungs, heart, kidney and brain, leading to hypertension, abdominal pain and different types of cancers. The concentration of essential and non-essential heavy metals in medicinal herbs beyond permissible limit is a matter of great concern to public safety all over the world [3]. World Health Organization (WHO) recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals. Contamination of agricultural soils with heavy metals has always been considered a critical challenge in scientific community. Heavy metals are generally present in agricultural soils at certain levels. Due to their cumulative behaviour and toxicity, however, they have a potential hazardous effect not only on crop plants but also on human health. Among the heavy metals cadmium, mercury, lead, arsenic, a non-essential toxic elements are of special concerns because of their potential toxicity at low concentrations. WHO recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals; further, it regulates maximum permissible limits of toxic metals like arsenic, cadmium, and lead, which amount to 1.0, 0.3, and 10 ppm, respectively. Plants can contain heavy metals from their presence in the soil, water, or air. High levels of toxic metals can occur in medicinal preparations when they are used as active ingredients, as in the case of Pb and Hg in some Chinese and Mexican medicines or when the plants are grown in polluted areas, such as near roadways or metal mining and smelting operations [4]. Environmental pollutants are toxic substances that enter the environment from both anthropogenic and natural sources. Usually, environmental toxicants include heavy metals and pesticides, and threaten the entire ecosystem, seriously damaging its function and structure. Heavy metals arise from many sources, such as industry, mining, and agriculture. In terms of the sources in the agricultural sector, these can be categorized into fertilization, pesticides, livestock manure, and waste water. Recently, the risk of heavy metals pollution in the environment has been increasing rapidly and creating turmoil, especially in the agricultural sector, by accumulating in the soil and in plant uptake. The heavy metals contamination problem has become urgent, and needs radical and practical solutions to reduce the hazards as much as possible. Heavy metal accumulation can be described as an aggregation of elements in the ecosystem. Plant roots are the essential point of contact for heavy metal ions transmitted from the soil. The mechanisms of heavy metals transmission to plants include (i) phytoextraction (ii) phytostabilization and (iii) rhizofiltration. These metals cause damage to plants, and extend to harm human health through transference in the food chain. Scientists have defined pesticides as synthesized chemical compounds used in many areas, including in the agricultural sector, to control pests. Therefore, pesticides are considered as efficient, economical, and effective weapons in integrated pest management systems (IPMs). The uncontrolled use of pesticides causes their bioaccumulation in food chains, which leads to high risk to mammals and other non-target organisms. In addition, the direct or indirect effect of pesticides on non-target organisms leads to an imbalance of the surrounding ecosystem. Moreover, the pesticide residues remain in the plant parts, soil, air, and even penetrate into water [5]. The soil-to-plant transfer of heavy metals is a very important step in the trophic transfer of such metals in food chains. These metals are taken up by plants from polluted soil and subsequently transferred to herbivorous animals along the food chain. Regarding contamination of the human food chain, contamination of crops such as cereals and vegetables is a very serious issue. Consumption of cereals contaminated with toxic heavy metals may cause risk to human health. The uptake and bioaccumulation of heavy metals in biota depend on several factors. For example, the uptake of heavy metals in plants depends on bioavailability of the metal in soil, which in turn depends on several factors such as metal speciation, pH, and organic matter contents in soil. Metals which are more bioavailable in soil may be accumulated in plants more easily and thus will have more bioaccumulation potential. An assessment of bioaccumulation of heavy metals in plants may be used for an estimation of bioavailability of the metals in soil. Such an assessment may also be used for knowing the contamination status of the environment. It has been reported that plants seem to be more sensitive to environmental changes than soils [6]. Recent concerns regarding the environmental contamination have initiated the development of appropriate technologies to assess the presence and mobility of metals in soil, water, and wastewater. Presently, phytoremediation has become an effective and affordable technological solution used to extract or remove inactive metals and metal pollutants from contaminated soil. Phytoremediation is the use of plants to clean up a contamination from soils, sediments, and water. Phytoremediation takes the advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant degradation abilities of the entire plant body. Phytovolatilization process is the plants ability to absorb and subsequently volatilize the contaminant into the atmosphere. This process is for metal contaminants in groundwater, soils, sediments, and sludges medium [7]. Traditional laboratory analyses of heavy metals in soils, such as Atomic Fluorescence Spectrometry (AFS), Atomic Absorption Spectrometry (AAS), and Inductively Coupled Plasma Optical

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Emission Spectroscopy (ICP-OES), are time consuming, laborious, and expensive. Some other methods such as portable X-ray fluorescence (PXRF), Laser-induced Breakdown Spectroscopy (LIBS), hyperspectral, visible-near infrared spectroscopy (Vis-NIR), and mid-infrared spectroscopy (MIR) have been considered as rapid, effective techniques to measure total concentrations of heavy metals in soil [8]. Many anthropogenic activities affect soil and the effectiveness of the plant, like urbanization, smelting, sludge, military operations, mining, dumping, and excess amounts of pesticide and insecticide applications. These activities inhibit the growth of the plants and cause many toxic symptoms in the plants due to heavy metal contamination, and these metals destroy human health by entering into the food chain. Crop yield and productivity can be improved and the health risk decreased acutely by removing the harmful effects of these heavy metals [9]. In developing countries, agricultural land is contaminated by heavy metals, which is a serious environmental problem due to heavy metal toxicity. Irrigation water contaminated by industrial effluents has led to severe heavy metal contamination in soil and plants. Due to the use of wastewater for soil irrigation, the concentration of heavy metals in the edible parts of growing plants is increasing. Pollution due to heavy metals poses an environmental threat and is currently a major concern. Rapid industrial development has resulted in a serious concern for natural resources such as soil and water in many countries. Industries are one of the anthropogenic activities that have contributed to increased concentrations of many heavy metals in the environment [10].

Materials and methods

Procurement of formulation

Formulation of *Amartottara Kwatha* churna (AK) was prepared by the combination of *Zingiber officinale*, *Tinospora cordifolia* and *Terminalia chebula* in the ratio of (2:6:4). Rhizome of *Zingiber officinale,* dried stem of *Tinospora cordifolia* and fruit pericarp of *Terminalia chebula* were collected from plants of local area and authenticated from CSIR-NISCAIR Delhi. The drugs were sun dried, coarsely powdered and stored in airtight containers. All chemicals and reagents used were of LR grade. Two marketed Ayurvedic formulations of *Amartottara Kwatha* namely Ashtavaida tradition *Amartottara Kwatha* (AAK) and Kerala Ayurveda *Amartottara Kwatha* (KAK) were purchased from local market. These two marketed products are the formulations of *Amartottara Kwatha* with two different brand names. AK, AAK and KAK are the formulation abbreviation used in this article.

Sample preparation

Heavy metals and pesticides analysis were carried out according to guidelines of the Association of Official Analytical Chemists (AOAC) and Ayurvedic Pharmacopoeia. For heavy metal analysis, 5 ml 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. Analysis was done using atomic absorption spectrophotometer (AA6300,

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Shimadzu, Japan). The standard reference material of all the metals Pb, Cd,Cr and Ni were used for calibration and quality assurance for each analytical batch. The efficiency of digestion of plant samples was determined by adding standard reference material of metals to different samples. After addition of standards, samples were digested, and metals were estimated as described above. Three replicates were analyzed to assess precision of the analytical techniques, and results were averaged. The proposed method for estimation of heavy metals was validated for linearity, recovery, reproducibility, limit of detection (LOD) and limit of quantification (LOQ).

For determination of pesticide residues, 2 g of each sample was mixed with 150 ml hexane and extracted in Soxhlet apparatus. From hexane extract traces of water and oil were removed. After oil removal, this extract was concentrated on rotary evaporator under reduced pressure and this concentrated extract was transferred to clean-up column. Carefully the Elute was collected and made up to 5 ml with hexane. Aliquots of above concentrate were injected into precalibrated GC machine equipped with 63Ni electron capture detector. Operation temperature was programmed at 200◦C, 195◦C, 220◦C for injector, column, and detector, respectively. Purified nitrogen gas as carrier gas was used at flow rate of 60 ml/min. Limit of detection was 0.1 to 0.5 ppb for organochlorine pesticides analyzed. For checking cross contamination procedural blanks were used. Recovery studies with purified samples indicated that overall recovery value exceeded 80%. Identification and quantification were accomplished using known amount of external standard procured from Sigma–Aldrich [11].

Instrument condition for CHNS analysis by CHNS/O analyser: CHNS analyzer, (Model FLASH 2000 CHNS/O Analyzers Thermo Fisher Scientific Instruments) was used for determining the total carbon, hydrogen, nitrogen and sulphur. For the CHNS analysis, freeze-dried and crushed samples were weighed (5–10 mg) and mixed with an oxidizer (vanadium pentoxide) in a tin capsule, which is then combusted at 1 000 ˚C in a reactor. The sample and container melt, and the tin promote a violent reaction (flash combustion) in a temporarily enriched oxygen atmosphere. The combustion products CO_2, SO_2, and NO_2 were carried by a constant flow of carrier gas (helium) that passes through a glass column packed with an oxidation catalyst of tungsten trioxide and a copper reducer, both kept at 10000 ˚C. At this temperature, the nitrogen oxides reduced to N_2. The N_2, CO_2, and SO_2 were then transported by the helium to and separated by, a 2-m-long packed column [12].

Table 1: Heavy metals concentration (mg/kg) in different formulations of *Amartottara Kwatha*

S. No.	Heavy metals	Limits	Results of different formulations			Units
			AK	AAK	KAK	
1.	Arsenic (Ar)	3.0	0.01	<0.0005	<0.0005	mg/kg
2.	Cadmium (Cd)	0.3	0.03	<0.0005	0.001	-
3.	Lead (Pb)	10	0.6	0.2	<0.0005	-
4.	Mercury (Hg)	0.1	<0.0002	<0.0002	<0.0002	-

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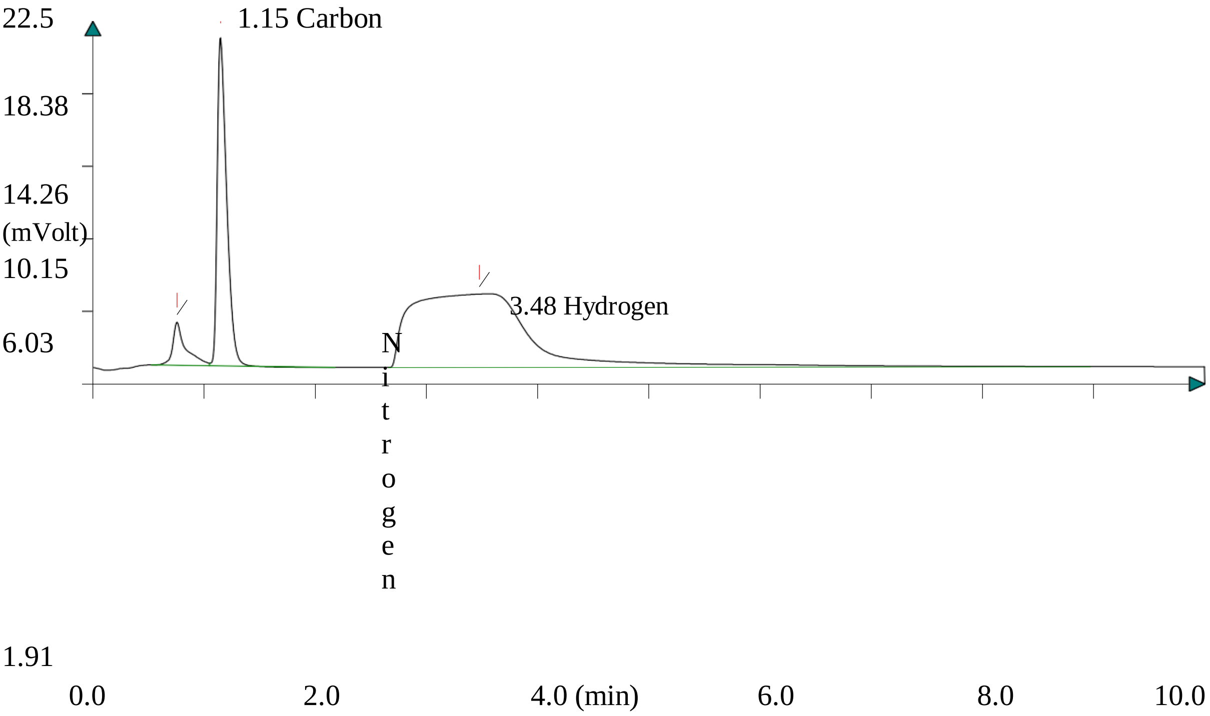
Table 2: Pesticide residue concentration (mg/kg) of KAK, AAK, AK

S No.	Substances	Units	Limits as per USP <561>	Results of different formulations		
				KAK	AAK	AK
1.	Acephate	mg/kg	0.1	BDL	BDL	BDL
2.	Alachlor	mg/kg	0.05	BDL	BDL	BDL
3.	Aldrin and dieldrin (Sum of)	mg/kg	0.05	BDL	BDL	BDL
4.	Azinophos-ethyl	mg/kg	0.1	BDL	BDL	BDL
5.	Azinophos-methyl	mg/kg	1	BDL	BDL	BDL
6.	Bromophos-ethyl	mg/kg	0.05	BDL	BDL	BDL
7.	Bromophos-methyl	mg/kg	0.05	BDL	BDL	BDL
8.	Bromopylate	mg/kg	3	BDL	BDL	BDL
9.	Chlordane (Sum of cis-, trans, and oxychlodane)	mg/kg	0.05	BDL	BDL	BDL
10.	Chlorfenvinphos	mg/kg	0.5	BDL	BDL	BDL
11.	Chlorpyriphos-ethyl	mg/kg	0.2	BDL	BDL	BDL
12.	Chlorpyriphos-methyl	mg/kg	0.1	BDL	BDL	BDL
13.	Chlorthal-dimethyl	mg/kg	0.01	BDL	BDL	BDL
14.	Cyfluthrin(sum of)	mg/kg	0.1	BDL	BDL	BDL
15.	λ –Cyhalothrin	mg/kg	1	BDL	BDL	BDL
16.	Cypermethrin and isomers (sum of)	mg/kg	1	BDL	BDL	BDL
17.	DDT sDum of σ, ρ'-DDE, ρ, ρ'-DDE, σ, ρ' DDT, ρ ρ' DDT, σ, ρ'-TDE, ρ, ρ'-TDE	mg/kg	1	BDL	BDL	BDL
18.	Deltamethrin	mg/kg	0.5	BDL	BDL	BDL
19.	Diazinon	mg/kg	0.5	BDL	BDL	BDL
20.	Dichlofluanid	mg/kg	0.1	BDL	BDL	BDL
21.	Dichlorvos	mg/kg	1	BDL	BDL	BDL
22.	Dicofol	mg/kg	0.5	BDL	BDL	BDL
23.	Dimethoate and omethoate (sum of)	mg/kg	0.1	BDL	BDL	BDL
24.	Endosulfan (Sum of isomer and endosulfan sulphate	mg/kg	3	BDL	BDL	BDL
25.	Endrin	mg/kg	0.05	BDL	BDL	BDL
26.	Ethion	mg/kg	2	BDL	BDL	BDL
27.	Etrimphos	mg/kg	2.05	BDL	BDL	BDL
28.	Fenchlorophos (Sum	mg/kg	0.1	BDL	BDL	BDL

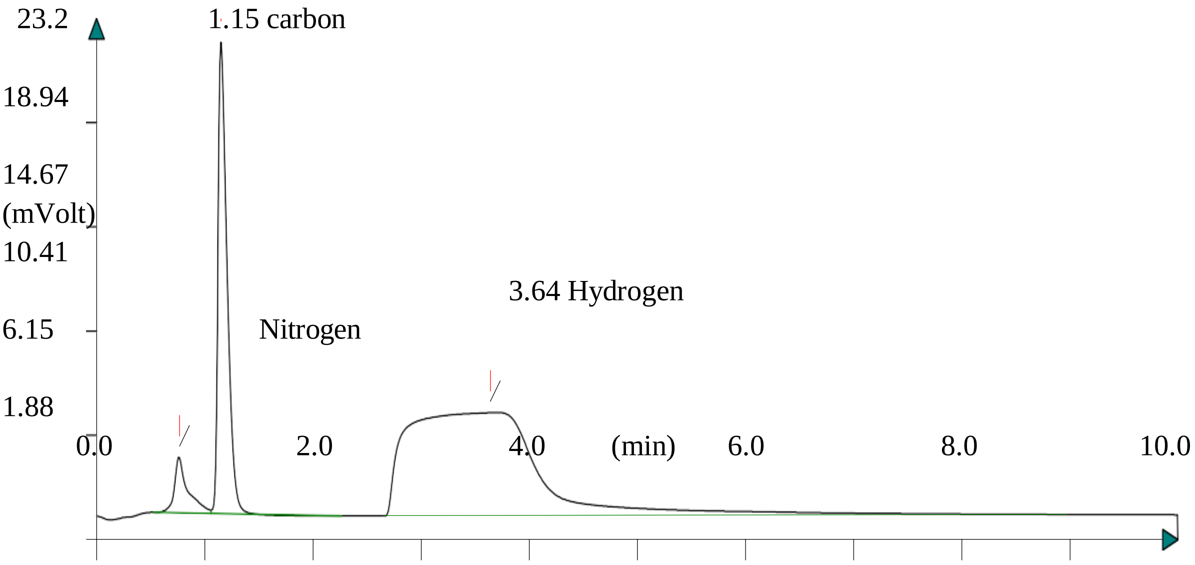
	of Fenchlorophos and Fenchlorophos oxon					
29.	Fenitrothion	mg/kg	0.5	BDL	BDL	BDL
30.	Fenpropathrin	mg/kg	0.03	BDL	BDL	BDL
31.	Fensulfothion (Sum of Fensulfothion oxon, Fensulfothion oxon sulfone, Fensulfothion sulfone)	mg/kg	0.05	BDL	BDL	BDL
32.	Fenthion (Sum of Fenthion, Fenthion oxon, Fenthion oxon sulfoxide, Fenthion sulfon and fenthion sulfoxide)	mg/kg	0.05	BDL	BDL	BDL
33.	Fenvalerate	mg/kg	1.5	BDL	BDL	BDL
34.	Flucythrinate	mg/kg	0.05	BDL	BDL	BDL
35.	T- Fluvalinate	mg/kg	0.05	BDL	BDL	BDL
36.	Fonophos	mg/kg	0.05	BDL	BDL	BDL
37.	Heptachlor (Sum of heptachlor, cis-heptachlorepoxide and trans-heptachlorepoxide)	mg/kg	0.05	BDL	BDL	BDL
38.	Hexachlorbenzene	mg/kg	0.1	BDL	BDL	BDL
39.	Hexaclorocyclohexane (Sum of isomers α-, β-, δ-, ε)	mg/kg	0.3	BDL	BDL	BDL
40.	Lindan (γ hexachlorocyclohexane)	mg/kg	0.6	BDL	BDL	BDL
41.	Malathion and Malaoxon (sum of)	mg/kg	1	BDL	BDL	BDL
42.	Macarbam	mg/kg	0.05	BDL	BDL	BDL
43.	Methacriphos	mg/kg	0.05	BDL	BDL	BDL
44.	Methamidophos	mg/kg	0.05	BDL	BDL	BDL
45.	Methidathion	mg/kg	0.2	BDL	BDL	BDL
46.	Methoxychlor	mg/kg	0.05	BDL	BDL	BDL
47.	Mirex	mg/kg	0.01	BDL	BDL	BDL
48.	Monocrotophos	mg/kg	0.1	BDL	BDL	BDL
49.	Parathion-ethyl and paraoxon-etyl (sum of)	mg/kg	0.5	BDL	BDL	BDL

50.	Parathion-methyl and paraoxon-metyl (sum of)	mg/kg	0.2	BDL	BDL	BDL
51.	Pendimethalin	mg/kg	0.1	BDL	BDL	BDL
52.	Pentachloranisole	mg/kg	0.01	BDL	BDL	BDL
53.	Permethrin and isomer (sum of)	mg/kg	1	BDL	BDL	BDL
54.	Phosalone	mg/kg	0.1	BDL	BDL	BDL
55.	Phosmet	mg/kg	0.05	BDL	BDL	BDL
56.	Piperonyl butoxide	mg/kg	3	BDL	BDL	BDL
57.	Pirimiphos-methyl (Sum of Pirimiphos-methyl and N-desethyl Pirimiphos-methyl)	mg/kg	4	BDL	BDL	BDL
58.	Procymidone	mg/kg	0.1	BDL	BDL	BDL
58.	Procymidone	mg/kg	0.1	BDL	BDL	BDL
59.	Profenophos	mg/kg	0.1	BDL	BDL	BDL
60	Prothiophos	mg/kg	0.05	BDL	BDL	BDL
61.	Pyrethrum (sum of cinerin I, Cinerin II, jasmolini, jasmolin II, pyrethrin I, pyrethrin II)	mg/kg	3	BDL	BDL	BDL
62.	Quinalophos	mg/kg	0.05	BDL	BDL	BDL
63.	Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorophenyl sulfide)	mg/kg	1	BDL	BDL	BDL
64.	Tecnazene	mg/kg	0.05	BDL	BDL	BDL
65.	Tetradifon	mg/kg	0.2	BDL	BDL	BDL
66.	Vinclozolin	mg/kg	0.3	BDL	BDL	BDL

Element analysis (CHN) curves of AAK:



Element analysis (CHN) curve of KAK:



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Element analysis (CHN) curves of AK

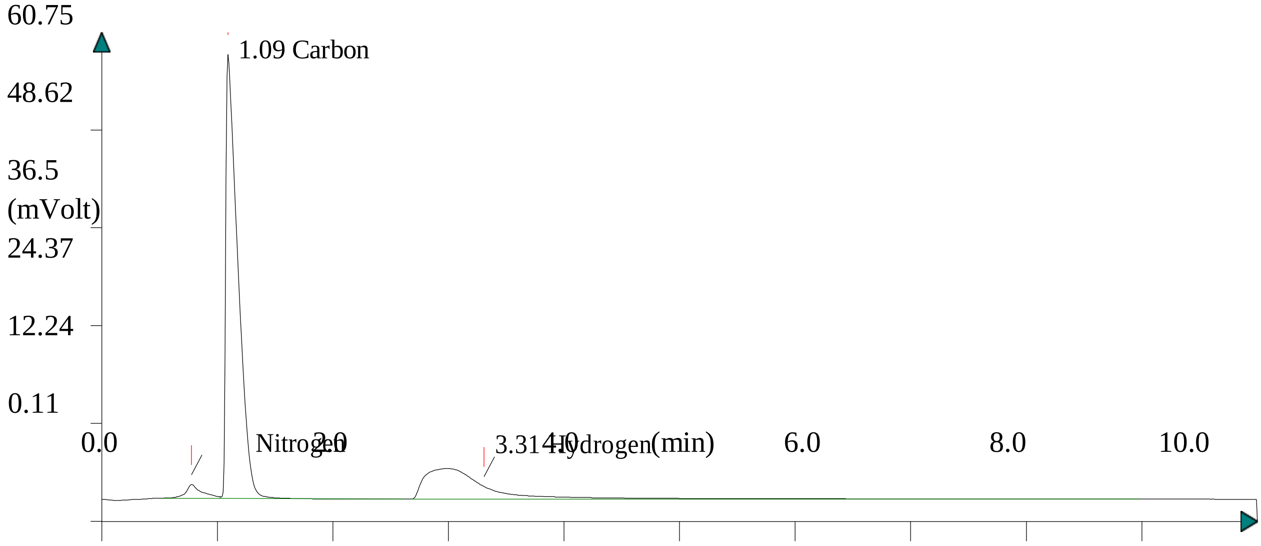

Table 3: Elemental analysis data of KAK, AAK, AK

Peak No.	Result of CHN analysis of AAK				Result of CHN analysis of KAK				Result of CHN analysis of AK			
	Retention time(min)	Area	Element %	Component	Retention time(min)	Area	Element %	Component	Retention time(min)	Area	Element %	Component
1.	0.758	199762	1.683	Nitrogen	0.767	196357	1.460	Nitrogen	0.775	164710	1.233	Nitrogen
2.	1.150	1090554	8.913	Carbon	1.150	1123362	8.308	Carbon	1.092	3999921	41.881	Carbon
3.	3.475	3174583	10.006	Hydrogen	3.642	3619747	10.334	Hydrogen	3.308	1463388	5.843	Hydrogen

Result: Metals like mercury, lead, cadmium, arsenic were estimated in *Amartottara Kwatha* (AK) and its two different formulations. Ar was found in *Amartottara Kwatha* (AK) that is mg/kg and Cd 0.03 mg/kg. Hg was detected in minimum detection limit. Pb was estimated in the amount of 0.6 mg/kg. In Ashtavaidya *Amartottara Kwatha* Pb was found to be 0.2 mg/kg. In Kerala Ayurveda *Amartottara Kwatha* Pb was found in minimum detection limit. Different Pesticides in *Amartottara Kwatha* were detected in below detection limit. Theresult of this study revealed that the values reported here are below the recommended levels. Therefore, content of mentioned heavy metals in selected samples may not be considered a critical issue. WHO/FDA has given the permissible limits of arsenic, mercury, lead, and cadmium in herbal drugs, i.e., 10, 1, 10, and 0.3 ppm, respectively. The results of the present study show that examined herbs for making in powder are subjected to contamination of the environment by heavy metals and the levels of these heavy metals were within permissible limits. It has been concluded from this study that estimation of heavy metals is highly essential for raw drugs used for the preparation of compound formulations. The result of

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elemental analysis obtained by CHNS/O analyser shows the presence of CHN in all formulations of *AMARTOTTARA KWATHA* churna.

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