

RESEARCH ARTICLE

Determination of pesticide residues and heavy metals in *Adhatoda Vasaka* Linn.

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Abstract: Heavy metals and pesticide residue analysis plays an important role in the quality control of medicinal plants like Vasaka (*Adhatoda vasica*). Hence a study was conducted to determine heavy metals and pesticide residues in this medicinal herb, which is a highly useful commodity in the health system. The reliable, rapid and nontoxic sample preparation method like QuEChERS and analytical methods like GC-MS were proposed for the simultaneous determination of pesticide residues and Heavy metal detection was carried out by ICP-MS. In this study, the presence of organophosphorus and organochlorine pesticides like alachlor, heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, etc. was checked but not detected. Heavy metals like Arsenic (As), Cadmium (Cd), Mercury (Hg) and Lead (Pb) are traced in samples about 2.3005 ppb, 0.799 ppb, 2.290 ppb and 10.204 ppb respectively in the present study.

Keywords: *Adhatoda vasica*/Vasaka, heavy metals, pesticide residues, ICP-MS, QuEChERS, GC-MS

1 Introduction

Herbal medicine was the first medicine method practised by Humans [1]. The World Health Organization (WHO) estimates up to 80 per cent of people still rely mainly on traditional remedies such as herbs for their medicines [2–5]. Nowadays, herbs are widely used in several applications around the world, such as in raw form, in pharmaceutical products, in food and cosmetic additives and as folk medicines [2, 6]. So, the quality of herbal drugs should not be compromised. Herbal drugs and their preparations are liable to contain various chemical contaminants. Pollution, environment, atmosphere, soil harvesting and handling are some factors, which play a major role in contamination of herbal plants [7]. Pesticide residues and heavy metals are types of toxic chemical contaminants of herbal drugs.

Heavy metals like arsenic (As), lead (Pb), mercury (Hg) and cadmium (Cd) are present in herbal plants which are not essential but harmful for the human body [8, 9]. Heavy metal contamination in herbal plants can be caused by environmental conditions, pollution, soil, water and the use of chemicals [7]. Just like heavy metals, pesticides are also hazardous chemicals for the human body as well as for herbal plants. The contamination of herbal plants by pesticides may be a consequence of the use of pesticides during cultivation, migration from neighbouring cultures or due to environmental contamination [10]. Literature suggests the availability of many articles proving the presence of both chemical contaminants in herbal plants [11–17]

Adopting the concern of these highly toxic contaminants of herbal plants which may lead to serious health issues for living beings, our objective of the present work was the determination of heavy metals like arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd) and detection of pesticide residues like alachlor, heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, DDT, DDE pesticides in Vasaka leaves (*Adhatoda vasica*). The Vasaka leaves (*Adhatoda vasica*) are used in respiratory problems like cough, asthma, bronchitis, tuberculosis and other disorders. In *Ayurveda*, it is an important drug prescribed for malarial fever, fever caused by *pitta* and *kapha* dosha, chronic fever, intrinsic haemorrhage, leprosy, skin diseases and piles [18]. The leaves of *Adhatoda vasica* have been found to contain primary alkaloid constituents like vasicine and vasicinone which are well-established as therapeutic respiratory agents [19].

Inductive Coupled Plasma Mass Spectroscopy (ICP-MS) is the widely used technique for heavy metal analysis [20]. So, in the present study, the concentration of heavy metals in Vasaka leaves (*Adhatoda vasica*) was analysed by using Inductive Coupled Plasma Mass Spectroscopy

(ICP-MS). Analysis of pesticide residues in herbs encounters certain difficulties. New trends in pesticide residue analysis are focussing on the miniaturization of the sample preparation method, leading to the development of fast, cost-effective, and environment-friendly procedures, such as quick, easy, cheap, effective, rugged and safe- QuEChERS method for routine use in laboratories [21, 22]. After the sample preparation, the analyte was analysed by GC-MS instrument which is the most widely used technique for qualitative and quantitative analysis of pesticide residues. Therefore, the present study was carried out by using the simplified sample preparation method QuEChERS and GC-MS for quantifying pesticides in Vasaka leaves.

2 Materials and methods

2.1 Sample material

A sample of Vasaka-IPRS/01/17 (*Adhatoda vasica*) was obtained from the Indian Pharmacopoeia Commission, Ghaziabad, Uttar Pradesh, India.

2.2 Chemicals and reagents

Analytical grade nitric acid and milli-Q water were used in the heavy metal analysis. For pesticide residue analysis, all pesticide standards were of more than 98% purity and purchased from Sigma Aldrich, Germany. Chromatography grade solvents (ethyl acetate and water) and analytical grade reagents (anhydrous magnesium sulphate – MgSO_4 , sodium sulphate, sodium chloride, sodium citrate dibasic, sodium citrate tribasic, and carbon black) were purchased from Merck India Ltd., Mumbai, India. C18 sorbent was purchased from Agilent Technologies, USA.

2.3 Sample preparation for heavy metal analysis

About 0.5-1.0 g of sample was crushed and homogenized in a microwave digestion vessel in which 3.0 ml of high purity nitric acid (Heavy-metal free) was added and placed in the microwave digestion vessel into the designated space of the microwave digester rotor. The rotor was locked and fit into the microwave digester. Digestion was performed as per parameters: For power, ramp Power is 800, Run time is 15.00 min, run level is 1 and for power, hold power is 800, run time 20 min. Excess nitrous oxide fumes were removed by putting the microwave digestion vessel in a water bath under exhaust till the brown fumes disappeared. The solution was cooled and transferred into a 15.0 ml PTEF centrifuge and volume was made up to 5 to 10 ml as per requirement. The solution was filtered using a syringe ($0.45\mu\text{m}$) filter. The sample was analysed by Inductive coupled plasma Mass Spectroscopy (ICP-MS). The reference solutions were prepared from 1 to 50 ppb.

2.4 Sample preparation for pesticide residue analysis

2.4.1 Extraction

Extraction was carried out by using a modified version of the QuEChERS (quick, easy, cheap, effective, rugged and safe) method. Where 2 g of the homogenised dry herb sample of vasaka leaves was weighed into a 50 ml centrifuge tube. The sample was dissolved in 10 ml of Milli-Q water/ HPLC grade water and vortexed for 2 min. After that 10 ml of ethyl acetate was added to this centrifuge tube and vortexed for 2 min. A buffer-salt mixture of 4 g anhydrous MgSO_4 + 1 g of sodium chloride and 1 g sodium citrate dibasic + 0.5 g sodium citrate tribasic was added for phase separation and pesticide partitioning. Then, the sample was vortexed for 10 min and again centrifuged at 10°C for 10 min, 8000 rpm. The supernatant (2 ml) was collected and preceded for cleanup treatment.

2.4.2 Cleanup

The cleanup procedure was done by using C18 sorbent. After centrifugation at 8000 rpm for 10 min as described in the extraction, 6 ml of supernatant was transferred to 15 ml centrifuge tubes containing 150 mg C18 along with 900 mg MgSO_4 and 45 mg carbon black. The extract was vortexed for 2 min and then centrifuged for 5 min at 10,000 rpm, 10°C . The supernatant (2 ml) was filtered by a syringe filter for final sample analysis. The final filtered solution was used for multi-pesticide residue analysis in GC-MS.

2.5 Preparation of pesticide standard solutions

Mixed standard stock solution (10 ppm)/($10\mu\text{g ml}^{-1}$) and working standard solutions ($10\text{-}200\text{ ppb}$)/ ($0.01\text{-}0.2\mu\text{g ml}^{-1}$) of different pesticides were prepared in HPLC grade ethyl acetate and

stored at -20°C ($\pm 1^{\circ}\text{C}$). The prepared standards must be used within 1 month.

2.6 Operating conditions of instruments

2.6.1 Apparatus and ICP-MS condition for heavy metal analysis

Thermo Fisher Scientific ICP-MS (ICAPQ Model) was used to assess the profiling of trace elements of heavy metals in the *Adhatoda vasica* sample. The operational conditions were: plasma forward power 1550 W, interference temperature 30°C , argon gas was used as a plasma gas, cool flow 14.0 l/min, auxiliary flow 0.8 l/min, nebulizer flow 1.06 l/min.

2.6.2 Apparatus and GC-MS conditions for pesticide residue analysis

For the detection and quantification of pesticide residues in Vasaka leaves, the sample was analysed on 7000C Triple Quadrupole GC-MS (Agilent Technologies). GC separation was performed with following operating conditions; initial oven temperature 70°C , held for 1 min, then $150^{\circ}\text{C min}^{-1}$ ramp to 250°C , held for 10 min and then 300°C , held for 15 min; carrier gas He at constant flow rate 1 ml min^{-1} , injection volume $1\ \mu\text{l}$ (splitless), the temperature for Inlet, Ion source and MS transfer line was 250, 230 and 280°C respectively, and total run time was 45 min.

3 Results

3.1 Heavy metal analysis

Heavy metals like Arsenic (As), Cadmium (Cd), Lead (Pb) and Mercury (Hg) are traced in the present study. The presence of these heavy metals was analysed in the Vasaka leaves and the results are tabulated in Table 1.

Table 1 Determination of heavy metals in Vasaka by ICPMS

Sr. No.	Sample Name	Arsenic (As)	Cadmium (Cd)	Mercury (Hg)	Lead (Pb)
1.	Vasaka (<i>Adhatoda vasica</i>)	2.3005 ppb	0.799 ppb	2.290 ppb	10.204 ppb

3.2 Pesticide residue analysis

Determination of pesticide residues in medicinal herbs is a difficult task since it contains various pigments and secondary metabolites. The pesticide residues were not detected in vasaka leaves. Figure 1 is the Vasaka sample chromatogram which indicates that there is no presence of pesticide residues and Figure 2 is the chromatogram of standard pesticide residues which were spiked in the Vasaka sample. The result analysis of the sample is given in Table 2.

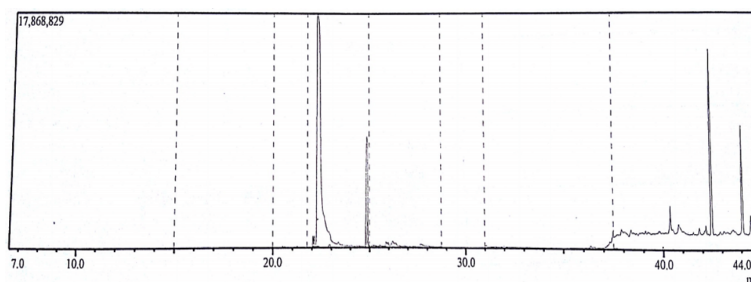


Figure 1 Chromatogram of Vasaka extract

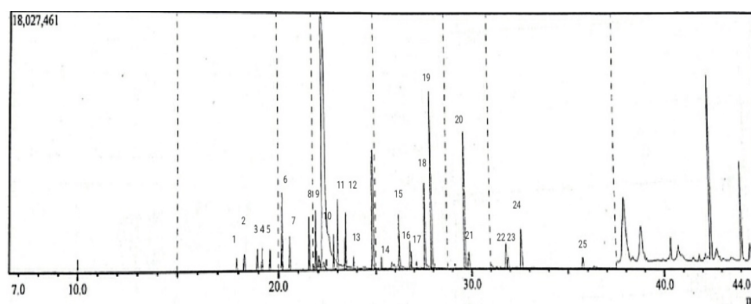


Figure 2 Chromatogram of standard pesticide residues with Vasaka extract

Table 2 Determination of pesticide residues in Vasaka by GC-MS

Sr. No.	Pesticide name	Category	Retention time (min)	Presence of Pesticides in Vasaka leaves
1	Phorate	Organophosphate	17.927	ND
2	Alpha HCH	Organochlorine	18.303	ND
3	Methyl Paraoxon	Organophosphate	20.189	ND
4	Delta HCH	Organochlorine	20.562	ND
5	Malaoxon	Organophosphate	21.532	ND
6	Alachlor	Phenyl amides	21.884	ND
7	Methyl Parathion	Organophosphate	21.869	ND
8	Fenitrothion	Organophosphate	22.787	ND
9	Malathion	Organophosphate	23.006	ND
10	Phorate sulfoxide	Organophosphate	23.427	ND
11	Chlorpyrifos	Organophosphate	23.386	ND
12	Aldrin	Organochlorine	23.828	ND
13	Phorate Sulfone	Organophosphate	23.425	ND
14	Heptachlor epoxide	Organochlorine	25.279	ND
15	Cis-chlordane	Organochlorine	26.220	ND
16	Alpha-Endosulfan	Organochlorine	26.832	ND
17	Trans chlordane	Organochlorine	26.777	ND
18	DDE	Organochlorine	27.563	ND
19	O, p – DDD	Organochlorine	27.908	ND
20	Dieldrin	Organochlorine	28.054	ND
21	Endrin	Organochlorine	29.121	ND
22	Beta- endosulfan	Organochlorine	29.632	ND
23	O, p – DDT	Organochlorine	29.655	ND
24	Endosulfan sulfate	Organochlorine	31.795	ND
25	Methoxychlor	Organochlorine	32.587	ND

Note: ND = Not Detected

4 Conclusion

Determination of the pesticide residues and heavy metals in herbs is essential to assess the safety to the consumers and avoid the chronic toxicity related to long-term use. For developing regulatory guidelines regarding the management of pesticide residues in herbal products and herbal plants, the development of simple as well as sensitive methods would be quite useful. Considering this, a rapid and sensitive method has been proposed for the extraction of multiclass pesticides in different popular medicinal herbs in India and the method standardized in the present study will also be of immense use in monitoring market samples of medicinal herbs to ensure quality and safety of the consumers worldwide. From this study, it has been concluded that by using the above method of pesticide residue analysis, given pesticide residues were absent in the Vasaka leaves (*Adhatoda vasica*) sample and the content of heavy metals in Vasaka leaves (*Adhatoda vasica*) complied with the standard limits of heavy metals as per the Ayurvedic Pharmacopoeia.

The Indian Pharmacopoeia Commission is working in this area to ensure the limits and importance of heavy metal and pesticide contaminants in medicinal plants.

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Conflicts of interest

There is no conflict of interest among the authors.

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