The Pharma Innovation Journal 2018; 7(11): 168-172

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(11): 168-172

www.thepharmajournal.com
Received: 21-09-2018
Accepted: 24-10-2018

Muhamad Ibrahim A
Vice President, R&D, R&D Centre, Sami Labs Ltd, Peenya Industrial Area, Bangalore, Karnataka, India

Nagarajan A
Principal Scientist, R&D, R&D Centre, Sami Labs Ltd, Peenya Industrial Area, Bangalore, Karnataka, India

Muhammed Majeed
Founder & Managing Director, Sami - Sabinsa group of Companies, Peenya Industrial Area, Bangalore, Karnataka, India

Correspondence
Nagarajan A
Principal Scientist, R&D, R&D Centre, Sami Labs Ltd, Peenya Industrial Area, Bangalore, Karnataka, India

Analysis of heavy metals and mineral elements in *Morinda tinctoria* Roxb. *Vitex negundo* L. and *Enicostema littorale* Blume. Leaves extracts: A traditional wound healing plants

Muhamad Ibrahim A, Nagarajan A and Muhammed Majeed

Abstract
This study aims to validate the analytical method for the determination of the mineral elements in the leaves of ethanol extracts of *Morinda tinctoria, Vitex negundo* and *Enicostema littorale*, a traditional wound healing plants. Rapid and accurate analytical technique is required for elemental analysis and the technique, Inductively Coupled Plasma Mass Spectrometry, ICP-MS is one of the best technique available because of its extensive availability and ease of use. It is important to estimate the amount of minerals in this plants, particularly of heavy metals to find out whether the dosage of the botanical as food or drug violates the levels of admissible elements and heavy metals. Pb, AS, Cd, Hg and Na, K were determined by using ICP-MS. It is concluded that *Morinda tinctoria, Vitex negundo* and *Enicostema littorale* has enriched source of potassium and its amount of heavy metals present in the each extract is as per the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines. These extracts can be utilized for oral administration.

Keywords: *Morinda tinctoria, Vitex negundo* and *Enicostema littorale, Mineral elements*, ICP-MS

Introduction

Medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents that produce a definite physiological action on the human body [13]. In 1990, the Nutrition Libelling and Education Act mandated, for the first time, labels providing information about the nutritional content of nearly all processed foods [2, 17]. At the present time, the only trace element content required to be listed on the label is for the elements sodium, calcium, and iron. *Morinda tinctoria* has been reported to have antimicrobial, anticonvulsant, cytoprotective, antioxidant effects and wound healing activities [3, 6, 10, 12, 15, 20]. *Vitex negundo* leaves are traditionally used in alternate medical systems of India like Siddha, Ayurveda and Unani. Although the leaves have been claimed to have several therapeutic potentials, they are most important in wound healing and anti-inflammatory activities [7]. Therapeutic plants are assuming a huge role in relieving different human ailments from olden days onwards among them, *Enicostema littorale* Blume is one of the woody annual herbs of the family Gentianaceae. Mineral elements are essential for growth and well being.

Raw material herbs are easily polluted by metals and microbial growth due to factors such as environment, atmosphere, soil, harvesting and handling [4]. After collection and conversion into dosage form the heavy metals confined in plants enter into the human body and may disturb the normal functions of central nervous system, liver, lungs, heart, kidney and brain, leading to hypertension, abdominal pain, skin eruptions, intestinal ulcer and different kinds of malignancies. Hence, quantifying the elements and heavy metals present is important not only in raw material but also in the extracts too. However, United States Pharmacopeial Convention (USP) recommends the use of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to measure the levels of elemental impurities in drug products and ingredients. ICP- MS is an appropriate technique for characterizing a few high concentrations elements present in the final drug product and can be tested accurately determined with the technique even at too low amount also. It is a type of mass spectrometry which is capable of detecting metals and non-metals at concentrations as low as one part in 10 [15]. This is achieved by ionising the sample with ICP and then using a MS to separate and quantify those ones.

The present study attempt to validate the quantity of that mineral elements present in the leaves of ethanol extract of *Morinda tinctoria*, *Vitex negundo and Enicostema littorale* using the technique of ICP-MS to estimate the amount of heavy metals present in the each extract. Review of literature on these three plants ethanol extracts showed that there was no data available on the elemental composition. This is the first report on elemental composition and heavy metal content in the leaf extracts. ICP-MS is an ideal choice for the laboratory that is looking for the lowest promising detection limits and the maximum level of productivity. The technique is relatively free from interferences, rapid, accurate and reproducible when compared to the existing scientific tools such as Atomic absorption Spectroscopy etc.

2. Materials and Methods

2.1 Sample Collection

The plant specimens *Morinda tinctoria* leaves and *Vitex negundo* leaves were collected from Kallakurichi, Villupuram district, Tamil Nadu and healthy leaves of the *Enicostema littorale* were collected from Virudhunagar, Tamil Nadu, India during April 2013- June 2013. The voucher specimens (Ref. RRCBI-AP4665/66/67) were deposited at National Ayurveda Dietetics Research Institute (NADRI), Bangalore-560 011, India. The taxonomic identification was done by Dr. B.N. Sridhar, Assistant Director, (NADRI), Bangalore. The fresh leaves were washed with tap water, rinsed with distilled water, air-dried for an hour, washed and rinsed once again thoroughly in distilled water and shade-dried in open air. The dried leaves were then grinded and the coarse powder sieved in 20 mesh size sieve.

2.2 Extraction

1 Kg of the powdered leaf is extracted with 100% ethanol at Soxhlet apparatus at 60° - 65°C for 3h. The extraction was repeated three more times. The extracts of leaves were filtered through polypropylene cloth concentrated, dried in vacuum and the yield recorded. The extract obtained was used for elemental and heavy metal analysis. The ethanol extract of leaves of *Morinda tinctoria, Vitex negundo and Enicostema littorale* were used for this study.

2.3 Reagents

For sample digestion trace metals-grade concentrated HNO3 (69-70%, Fisher Scientific) was used. Double de ionized water (Milli-Q Millipore 18, 2 M cm-1 resistivity) was used for all dilutions. The element standard stock solutions, Lead (Pb), Arsenic (As), Cadmium (Cd), Mercury (Hg), Sodium (Na), Potassium (K) in 1000 mg L^{-1} used for calibration which were supplied by Merck.

2.4 Standard Preparation

An aliquot amount of standards were prepared using 10 ml volumetric flask. Lead, arsenic, cadmium as 1,2,4,10,20 µgkg^{-1}; mercury as 0.1, 0.2, 0.4, 1, 2 µg kg^{-1}; Potassium, sodium, as 1, 10, 25, 40, 50 µg kg^{-1}; Gold as 10 µg kg^{-1} (for mercury stabilization) prepared in 16% HNO3.Linearity studies were made by standard methods [8].

2.5 Sample preparation

0.5 gram of dry leaves extract of each *Morinda tinctoria, Vitex negundo* and *Enicostema littorale* was weighed into the digestion tube and then added with 8 ml of concentrated HNO3. Sample was pre-digested for 15 minutes. Microwave digestion of the samples was done using Mars 6 Microwave digestion system. The leaves extract was digested according to the following optimized program (Table 1). After cooling the whole digest was transferred into 50 mL standard volumetric flask and diluted to 50 mL with double deionized water. Reagent blanks were also prepared with same conditions.

Table 1: Programme - Microwave Digesting Parameters

S. No	Time (Min)	Temperature (°C)	Power (W)
1	0 to 15	0 - 130	600
2	15 to 25	Maintain 130	600
3	25 to 35	130 - 150	700
4	35 to 45	Maintain 150	700
5	45 to 60	Cooling	–

2.6 Quantitative elemental analysis

Plant extracts was digested by strong acid and analysed with Perkin Elmer ICP-MS, Nexion 350x; (Waltham, Massachusetts, U.S) Collision mode-with kinetic energy discrimination (KED) and quadrupole ion deflector. The software used is Syngistix. (Figure 1).

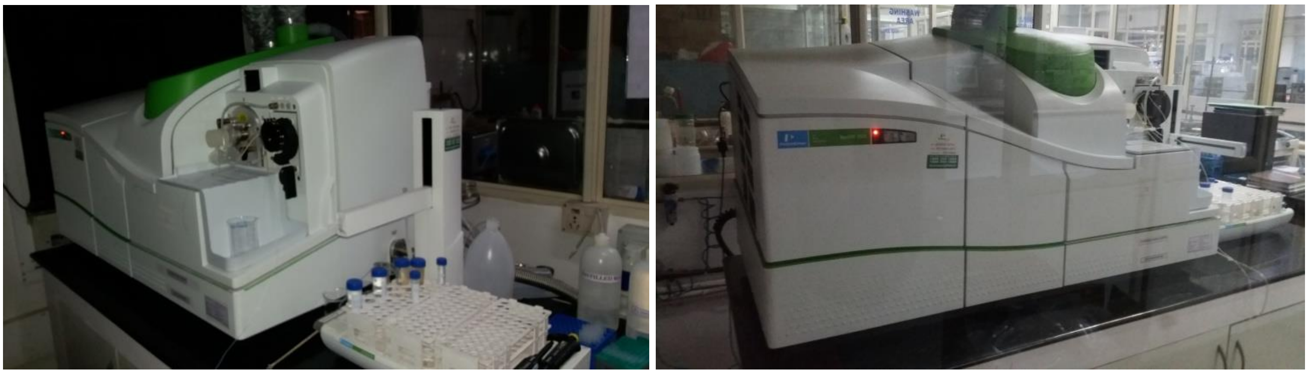


Fig 1: Photographs of ICP-MS

2.7 ICP-MS – Mechanism

Liquid sample is introduced in argon plasma as aerosol droplet. The plasma dries the aerosol, dissociates the molecules, then removes an electron from the components, forming singly charged ions which are directed to mass filtering device known as mass spectrometer. Ions enter MS (mostly quadrupole), they are divided by their mass to charge ratio and gets detected. ICP-MS parameters are mentioned in Table 2.

Table 2: Operating Parameters for ICP-MS

ICP-MS Parameter	Value
Plasma Gas Flow	18L Min^{-1}
Nebulizer Gas Flow	0.82 L Min^{-1}
RF Generator	1.6 KW, 27.12 MHz
Carrier gas	Argon
Mode	KED
Cell gas	Helium
Cell gas Flow	3 mL Min^{-1}
Sample Flush	35 Seconds
Read Time	15 Seconds

2.8 Quantification

The calibration curve was drawn with the help of software as intensity along Y axis *vs* concentration along X axis. The correlation coefficient (R^2) > 0.99. The sample solution was aspirated as unknown and the concentration was checked. If the concentration of the sample is above the calibration curve then the sample was diluted in such a way that it is within the range of calibration curve. The content of elements was calculated as below:

$$\text{PPM} = \frac{[\text{Sample concentration from Linearity graph– Process blank value}] \text{ X Sample dilution}}{\text{Sample weight (g) X 1000}}$$

$$\text{Calculation in Percentage of Elements present} = \frac{\text{PPM}}{10000}$$

2.9 Linearity

The range is possible to get proportional analytical results directly, to the analyte concentration over the range stipulated. The equation of the calibration curve that relates the net intensity (cps) and Concentration (ppb) is $y= mx+c$. The linear regression was used to estimate the coefficients of the calibration curve from one set of experimental measurements. The coefficient of correlation (R^2) value of 0.99 was used as the lower limit for setting the coefficients of the curve.

2.10 Limit of Detection (LOD)

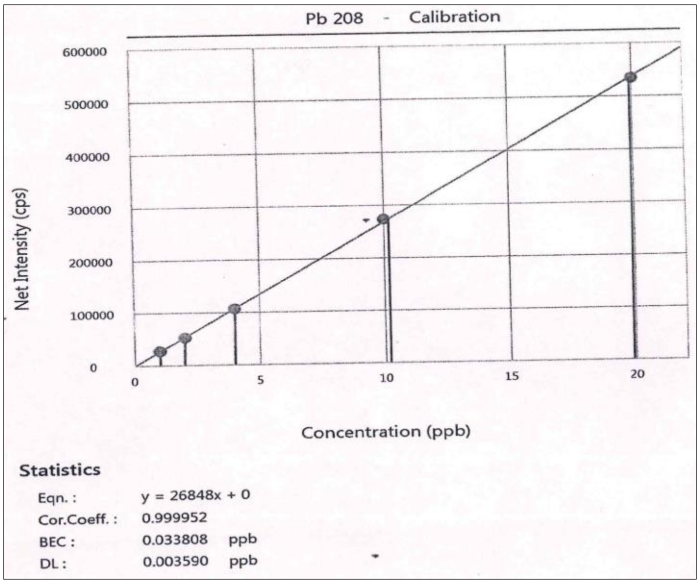
It is the lowest concentration at which the element can be distinguished (i.e. detected) from noise. A total of 10 repetitions were performed. The limit has been studied as three times Standard deviation (SD) using the formula: *LOD= 3 X SD.*

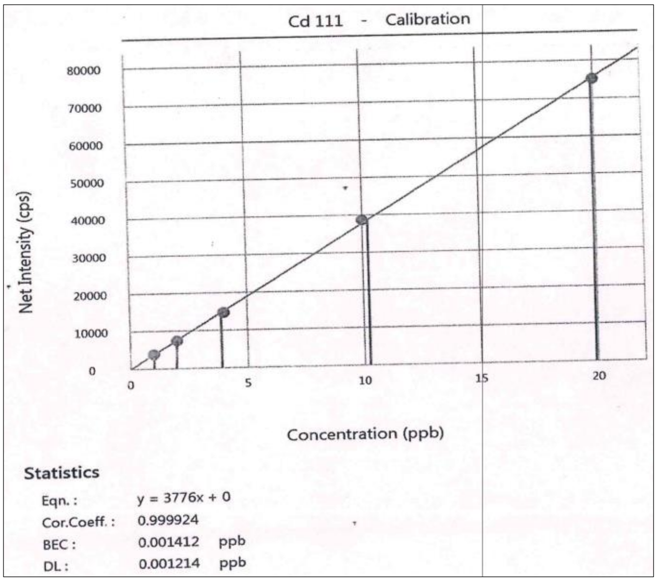
2.11 Limit of Quantification (LOQ)

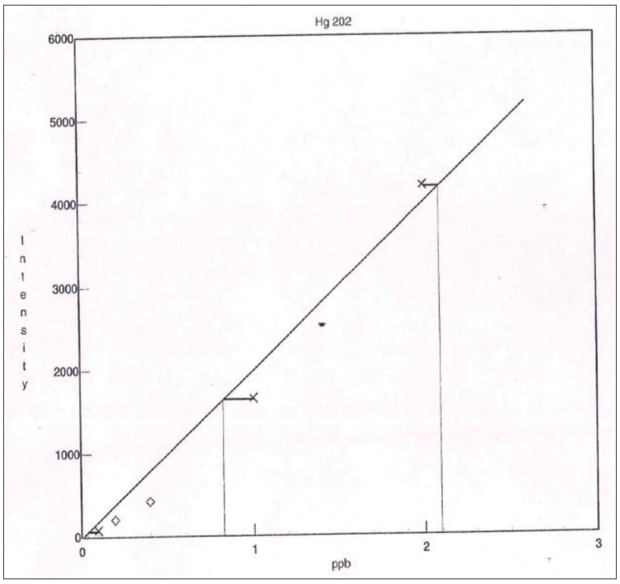
It is the lowest concentration of analyte in a sample that can be established with acceptable precision and accuracy. The LOQ was studied in the same way as LOD being set as ten times the SD, using the formula: *LOQ = 10X SD*

2.12 Calibration of standards by ICP-MS

The ICP-MS system was calibrated by the standards supplied by Perkin Elmer (Figure 2).







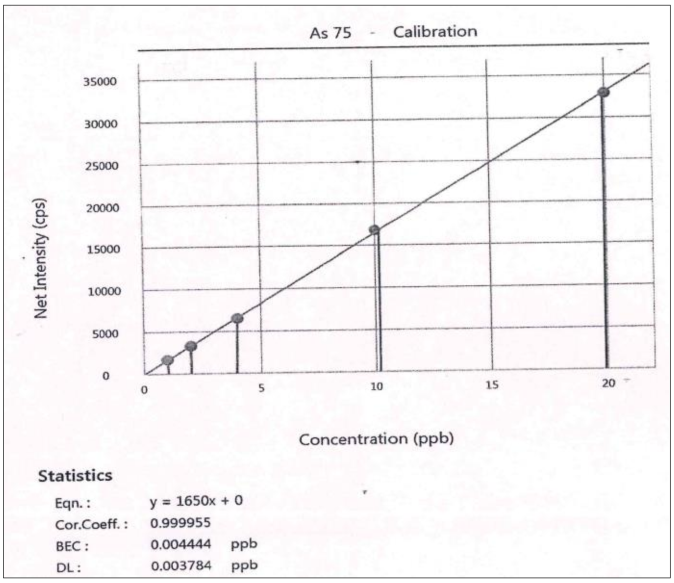


Fig 2: Linearity graph of standard of Pb, As, Cd, and Hg with correlation coefficient (R^2)>0999.

The isobaric spectral interferences originating from the polyatomic ion species involving the sample matrix elements may be eliminated by selecting a suitable isotope corrected or reduced by applying interference correction equations. The system calibration was done before starting the analysis of sample and the calibrated values are shown in Table 3

Table 3: Calibration

Standard	Apparent Conc. (ppb)	Net Intensity (cps)		
		Pb 208	**Cd 111**	**As 75**
Blank	-	908	5	7
Cal.STD.1	1	27620	3852	1718
Cal.STD.2	2	53195	7450	3319
Cal.STD.3	4	107002	14696	6534
Cal.STD.4	10	273836	38664	16812
Cal.STD.5	20	534385	75160	32857

2.13 Statistical analysis

The data was analysed by MS-EXCEL and results are expressed as means and standard deviation (Mean + SD)

3. Result and Discussion

Elements are found in environment and enter the human body via food chain including medicines and water leading to health issues. The excess heavy metals affect the stability of formulation and catalyse degradation of drug substance. The use of medicinal plants in therapeutics or as dietary supplements goes back beyond recorded history and has increased substantially in the last decades [11, 22]. The ethanol extract of leaves of *Morinda tinctoria, Vitex negundo and Enicostema littorale* yields were recorded and the details as follows: *Morinda tinctoria* leaf ethanol extract 18% (w/w), *Vitex negundo* leaf ethanol extract 12% (w/w) and *Enicostema littorale* leaf ethanol extract 20% (w/w). The result of present study also proves the extractability of *Vitex negundo.* Nirmalkumar (2014) reported alcoholic soluble extractive value 5.99% but we achieved 12% which indicates that the maximum extractability was achieved by this study design [16]. Pawar *et al.,* (2017) reported the content of sodium and potassium as 31.98 □g/g and 2332 □g/g in *Vitex negundo* leaves [18], We have found the content of sodium as 457 µg/g, and potassium to be very high as 20225 µg/g. The ICP-MS analysis study revealed the presence of major elements like Na, K found in maximum concentrations which are the quantity elements. Further, the same author [Pawar *et al.,* (2017)] reported that from the *Vitex negundo* leaves heavy metals like Pb, As, Cd and Hg is present in 0 µg/g, 0.26, 0.09 and 0.12µg/g respectively [18]. The soluble extractive value (yield %) was found maximum in ethanol extract of all the three leaves and hence ethanol extract is most effective for studying the promising activity of these plants. Elemental analysis showed the presence of trace elements in sufficient concentrations and traces of heavy and toxic metals. Lead is known to cause neurological disorders, anaemia, kidney damage, miscarriage, lower sperm count and hepatotoxicity in higher concentration [1, 14]. Heavy metals /trace elements such as Pb, Cd, As and Hg were quantified in sufficient concentrations. (Table 4). International Council for Hormonisation (ICH) has followed three preventive measures like Safety, Toxicity and acceptance level and also evolution of toxicity data for potential source, establishment of permitted daily exposure and development for design for control. The quantity of Cd and Pb was observed in very low quantity of *Enicostema littorale* raw material leaf powder about 0.3 µg/g and Mercury (Hg) was not detected in the leaf powder [19].

Table 4: Quantitative elemental analysis and the permitted daily exposure (PDE) limits for drug products, according to their route of administration

			Leaves Extract			Specification limits		
Class	**Elements**	**Analyte Mass**	***Morinda tinctoria* extract (µg/g)**	***Vitex negundo* extract (µg/g)**	***Enicostema* extract (µg/g)**	**ICH Q3D (µg/day) [9]**	**USP <232> (µg/day) [21]**	**Oral permitted daily exposure (PDE) (µg/day) [9]**
1	Lead	208	0.03	0.01	ND	4	1.5	5.0
	Cadmium	111	ND	ND	ND	0.5	0.5	5.0
	Arsenic	75	0.01	0.02	0.14	1.5	1.5	15

	Mercury	202	ND	ND	ND	0.5	0.5	40
-	Sodium	23	48.55	457	12598	NA	NA	NA
	Potassium	39	11804	20225	9714	NA	NA	NA

ND = Not Detected or Less than 0.001 ppm; NA = Not Applicable
Values are expressed by mean ± SD of three samples

4. Conclusion

ICP-MS technique was employed for the determination of the trace elements and elements like sodium and potassium in the ethanol extract of leaves of *Morinda tinctoria, Vitex negundo and Enicostema littorale*. The trace elements as well as Sodium and Potassium played a vital role in biochemical process in humans other than nutritional value. It can be concluded that, concentrations of analysed elements were in order of K>Na. Also heavy metals like Arsenic and lead content was found to be very less and within the required limit and Cadmium and Mercury were not detected in all the three extracts. Hence, these three extracts meet the permitted limits of elemental contents as per ICH guidelines. In this study, four components of heavy metals analysed which were not previously reported, and confirmed the high Potassium and negligible amount of Sodium present in *M. tinctoria* leaves extract. However, this content is comparable with *Morinda citrifolia* which is reported to have 6100 µg/g of Potassium content [5]. Hence, these three ethanol extracts can be administered for oral as well as topical applications.

5. Acknowledgment

Our grateful thanks are to Dr. S. Natarajan, Senior Advisor (R&D), Prof K.V. Krishnamurthy, Consultant R&D, Mr. C.N. Vinay Kumar, Analyst Analytical R&D, Sami Labs Ltd., Bangalore for their advice and support.

6. Conflict of Interest

Authors have no conflict of interest.

7. References

1. Anonymous. Toxicological Profile for Lead. Agency for Toxic Substances and Disease Registry ATSDR: U.S. Department of Health and Human services, Public Health Service, Atlanta, GA, United States, 2007.
2. AOAC. Methods of Analysis for Nutrition Labeling; AOAC; Arlington, VA, 1993, 3-26.
3. Deepti K, Umadevi P, Vijayalakshmi G, Polarao BV. Antimicrobial Activity and Phytochemical Analysis of Morinda tinctoria Roxb. Leaf Extracts Asian Pacific Journal of tropical Biomedicines. 2012, 1440-1442.
4. Dushenkov V, Kumar PBAN, Motto H, Raskin I. Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. Environmental Science and Technology. 1995; 29:1239-1245.
5. Gomathinayagam Subramanian, Brij Tewari B, Rekha Gomathinayagam. Phytochemicals, Heavy metals and N.P.K analysis of crude leaf extract of Noni (*Morinda citrifolia*). Asian Journal of Biochemical and Pharmaceutical Research. 2017; 1(7).
6. Ibrahim M, Nagarajan A, Majeed M. Evaluation of *in-vivo* wound healing activity of the extract and oleuropein from *Morinda tinctoria* Roxb. (Rubiaceae), International Journal of Advanced Research. 2017; 5:889-905.
7. Ibrahim M, Nagarajan A, Majeed M. Wound Healing Activity of the Ethanolic Extract and Agnuside Isolated from *Vitex negundo* L. Leaves, Journal of Pharmacognosy and Phytochemistry. 2018; 7(5):1823-1832.
8. ICH. Guideline Q3D on Elemental Impurities, EMA/CHMP/ICH/353369/2013, 2016.
9. ICH. Guidance for Industry, Q3D Guideline for Elemental Impurities. Available at: http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html. December 16, 2014.
10. Jayasinghe ULB, Jayasooriya CP, Bandara BMR, Ekanayake SP, Merlini Assante LG. Antimicrobial activity of some Srilankan Rubiaceae and Meliaceae. Fitoterapia. 2002; 73:424-427.
11. Khan IA, Allgood J, Walker LA, Abourashed EA, Schelenk D, Benson WH. Determination of heavy metals and pesticides in ginseng products. Journal of AOAC International. 2001; 84:936-939.
12. Kumarasan TP, Saravanan A. Anticonvulsant activity of *Morinda tinctoria* Roxb. African Journal of Pharmacy Pharmacology. 2009; 3:63-65.
13. Mohanta B, Chakraborty A, Sudarshan M, Dutta RK, Baruah M. Elemental profile in some common medicinal plants of India. Its correlation with traditional therapeutic usage. Journal of Radio analytical and Nuclear Chemistry. 2003; 258(1):175-179.
14. Mudipalli A. Lead hepatotoxicity and potential health effects. Indian Journal of Medical Research. 2007; 126:518-527.
15. Muthukumaran P, Elayarani M, Shanmuganathan P, Cholarajana A. Antimicrobial activities of *Cassia auriculata* L and *Morinda tinctoria* Roxb, International Journal of Research in pure and applied Microbiology. 2011; 1(2):9-12.
16. Nirmalkumar N. Pharmacognostic and phytochemical analysis of *Vitex negundo* L. International Journal of Innovative Research Science and Engineering Technology. 2014; 3:14501-5.
17. NFPA. NFPA Guide to Nutrition Labeling; National Food Processors Association: Washington, DC, 1994, 1-194.
18. Pawar S, Kamble V. Phytochemical screening, elemental and functional group analysis of *Vitex negundo* l. leaves, International Journal of Pharmacy and Pharmaceutical Sciences. 2017; 9(6):226-230.
19. Sathish Kumar R, Lakshmi PTV, Annamalai A. Estimation of biomass contents and phytoconstituent analysis of *Enicostemma littorale* blume. International Journal of Pharma and Bio Sciences. 2012; 3(3)B:506-522.
20. Srilakshmi JK, Meenaa V, Sriram S, Sasikumar C. Phytochemical screening and antimicrobial evaluation of *Morinda tinctoria* Roxb. against selected microbes, International Journal of Pharmaceutical Innovations. 2012; 2(2):1-7.
21. USP. Chapter <232> Elemental Impurities - Limits, Pharmacopeial Forum. 2016; 42(2).
22. Woods PW. Herbal healing. Essence. 1999; 30:42-46.