https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. (1119-5096) *Vol. 27 (September 2024); 2719-2727*

Research Article

Metal Detection in Ehretia Microphylla (Boraginaceae Family) By Using ICP-MS

Shweta Sain[1*], Dr. Manjul Mishra[2]

[1]Research Scholar, Department of Chemistry, Apex University, Jaipur, India. [2]Professor, Department of Chemistry, Apex University, Jaipur, India.*

ABSTRACT
Indian culture cannot be separated from traditional medicine. India has a long history of ethnobotanical study. Many great efforts have been put into isolating organic compounds; their inorganic components have received less focus. Certain Inorganic Elements have a significant role in numerous biological functions in humans. Many diseases are brought on by elements in excess or lack. Here, the profile of specific trace elements in Ehretia microphylla is examined. The herbal characteristics of this plant are revealed by the qualitative and quantitative examination of trace components in it. This study deals with detection of trace elements, found in Ehretia microphylla and reveals that they are very important source in combating human diseases. Various concentrations of important Trace Elements are being analyzed that are necessary for the metabolism of the human body. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is being used to detect the trace elements like Ca, Mn, Fe, Cr, Cu, and K in the leaves of Ehretia microphylla.

Keywords: Ethano botanical, Ehretia microphylla, Inductively Coupled Plasma Mass Spectrometry, trace elements.

**Author for correspondence: Email: sainshweta001@gmail.com*

Receiving Date: 10/07/2024 Acceptance Date: 20/08/2024

DOI: https://doi.org/10.53555/AJBR.v27i1S.1465

© 2024 The Author(s).
This article has been published under the terms of Creative Commons Attribution-Non-commercial 4.0 International License (CC BY-NC 4.0), which permits non-commercial unrestricted use, distribution, and reproduction in any medium, provided that the following statement is provided. "This article has been published in the African Journal of Biomedical Research"

INTRODUCTION

Ehretia microphylla is a medicinal shrub that belongs to the Boraginaceae Family. The arrangement of leaves is in bunches, cutting-edge obsolete[2]. Blossoms are undivided or expanded. Fruit is globose, and maturing natural products are caramel orange in color. A decoction of E. microphylla leaves is being utilized to cure cold and abdominal problems, escalate fertility, and for the treatment of looseness of the bowels and diarrhea[3]. Leaves act as anti inflammatory. From the leaves of E. microphylla, many essential metals are detected to fulfil the daily requirements of human health[4]. In this research paper,

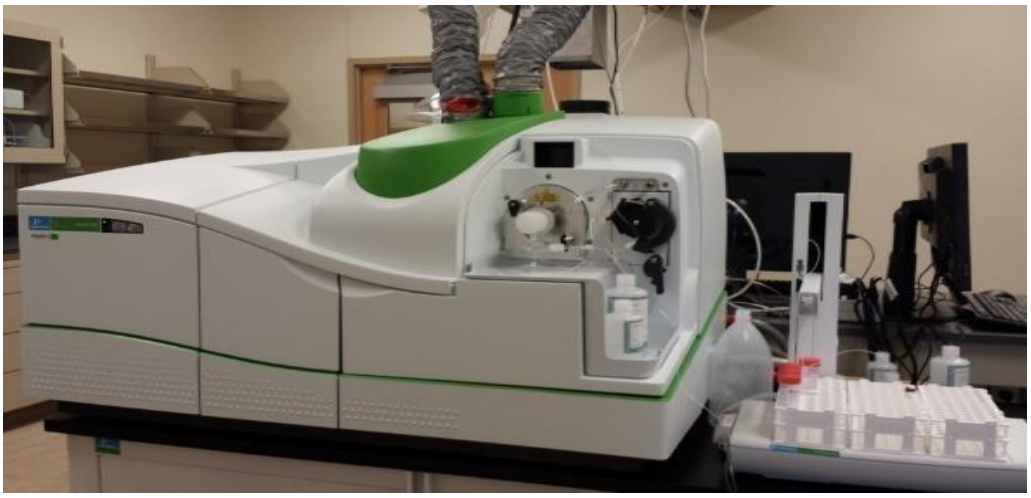


Fig.3.Inductively coupled plasma mass spectrometer [ICP-MS]

various metals are detected in Ehretia microphylla by the help of Inductively Plasma Mass Spectrometry, which finds wide medicinal applications. Metal levels in food are becoming increasingly important in terms of toxicological and nutritional perspective. Various techniques, including Atomic Emission Spectrometry (AES), Atomic Absorption Spectrometry (AAS), Induced Coupled Plasma Atomic Emission (ICP-AES), and Induced Coupled Plasma Mass Spectrometry (ICPMS), have been used to assess foods for various Elements[5-7]. Due to the special properties and authenticity of Inductively Coupled Plasma Mass

Spectrometry (Low Detection Limit, Multi-Element Capacity, Wide Linear Range, etc.), many research works dealing with the analysis of food samples by ICP-MS are being done in the recent years (Alam, Snow, & Tanaka, 2003[1] Nikkalinen & Mertanen, Roychoudhuri, Tokunage& Ando, 2003)[14]. Research works on medicinal importance of Ehretia microphylla are also being reported by V. Aarthi in 2012[15].

MATERIALS AND METHODS

Sample collection: After being cleaned, the samplesare firstly dried in the shade. The samples are thendried in an oven at 40^0 to 50^0 C until they have aconstant weight. Using a pestle and mortar, the drysamples are then pulverized and powdered. For furtherinvestigation, samples are labelled and kept in precleaned plastic vials.

Fig.1. Ehretia microphylla plant

Fig.2. Leaves of Ehretia microphylla

Chemicals and Reagents: • Diluent (2%v/v) HNO_3 & HCL Solution: Transfer Deionized Water (DI water) for Cleaning Purpose, each 10 ml of conc. HCl into a 500 ml volumetric 2004[10]; Perez Trujillo, Barbaste& Medina, 2002[13]; **Equipment/Apparatus:** • Micropipettes- fixed or variable, covering ranges • Inductively coupled plasma mass spectrometer- 20-200µL,100-1000µL. agilent number7800 • Bottles–polypropylene,100 and500M,

- Analytical balance
- Centrifuge tube-polypropylene,15,50 mL,
- Microwave digestion vessel-55 Ml liners and vent
- Syringe filter & Funnel, plugs,
- Argon Gas & Helium Gas
- Milestone,
- Elga Water System
- Volumetric flasks-polypropylene or poly-methyl pentane,

Millipore Water, Nitric Acid and Hydrochloric acid, flask, already containing 250 ml purified water. Mass spectrometer

tuning solution. Now total volume in the flask was made up to 500 ml by adding purified water and mixed well.

Solutions and Standards:

Tuning Solution: Concn.10 ppm; STD ID: NSL/S/ICP-0047; Purity-10 mg /ml.

Metal Standards Used

S. N.	Name of Standards	STD ID	Purity
1	Cr	NSL/S/ICP-0063	994mg/L
2	Cu	NSL/S/ICP-0033	99.9996%
3	Zn	NSL/S/ICP-0081	1001mg/L
4	K	NSL/S/ICP-0064	1001mg/L
5	Fe	NSL/S/ICP-0051	99.9955%
6	Ca	NSL/S/ICP-0030	99.9998%
7	Mn	NSL/S/ICP-0069	99.9998%

Table 1: Preparation of Standard Solutions:

Stock solution	Element/ STD ID	Conc. of STD (ppm)	Volume of STD(ml)	Make up Volume (ml)	Make up solution	Final concentration (ppm/ppb)
1	Cu [NSL/S/ICP-0033]	1000	0.1	10ml	Diluent	10 ppm
	Cr [NSL/S/ICP-0063]	10	1	10ml	Diluent	1 ppm
2	Mn 0069] [NSL/S/ICP-	1	0.1	10ml	Diluent	100 ppb
	Zn [NSL/S/ICP-0081]	000	0.1	10ml	Diluent	0 ppm
	K 0064] [NSL/S/ICP-	1000	0.1	10ml	Diluent	10 ppm
	Fe [NSL/S/ICP-0051]	000	0.1	10ml	Diluent	0 ppm
	Ca [NSL/S/ICP-0030]	1000	.1	10ml	Diluent	10 ppm

Table 2: Preparation of Calibration levels:

Calibration	Stock solution-1	Stock solution 2
Level 1	Diluent is used as a calibration blank	
Level 2	0.1 ml from 100 ppb	50µl
Level 3	50µl	100µl
Level 4	100µl	200µl
Level 5	250µl	300µl

Level 6	500µl	400µl
Level 7	100µl	500µl
The above each level (2 to 7) solutions were made up to 10 ml of each volumetric flask with diluents.		

The calibration level concentration of individual element in ppb from level 2 to level 7 are represented in Table 3.

Table 3: Calibration levels concentrations respectively in ppb

Elements	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7
Cr	1	5	10	25	50	100
Cu	1	5	10	25	50	100
K	50	100	200	300	400	500
Zn	50	100	200	300	400	500
Fe	50	100	200	300	400	500
Ca	50	100	200	300	400	500
Mn	50	100	200	300	400	500

Preparation of Standard Check Solutions: Calibration level 6 was used as standard check solution.

Preparation of Sample Solutions:

0.10205 g of dried and fine powdered leaf sample of Ehretia microphylla is weighed and transferred into a clean and dry beaker. 10 ml of conc. HNO3was added into the beaker and it was kept for pre digestion for 10 minutes to release the acid fumes. The beaker was capped and kept into hot plate for 10 minutes at 80 ºC. After the completion of open digestion, the beaker was allowed to attain room temperature. Now the sample solution was transferred into 50 ml volumetric flask.

The beaker was rinsed with two portions of 10 ml of water and rinsed portions were transferred into above 50 ml volumetric flask. Then the flask was diluted upto the volume with water. The solution was mixed well and filtered through 0.22 µm nylon syringe. This solution is named as Sample stock solution. If the solution concentration is found higher than linearity level then the sample stock solution is further diluted till it reaches to linearity level. This solution is called as Sample diluted solution-1. If the concentration of sample diluted solution-1 is still higher than the linearity level, then it is further diluted. This further diluted solution is now called as Sample diluted solution2 as shown in table 4.

Table 4: Preparation of Sample Diluted Solutions:

Name of Element	Sample diluted solution- 1			Sample diluted solution -2		
	Volume of sample stock solution (ml)	Make-up vol.(ml)	Make up solution	Volume of sample stock solution-1 (ml)	Make up vol(ml)	Make up solution
K	0.5ml	50ml	Water	-	-	-
Ca	0.5ml	50ml	Water	2ml	10 ml	Water

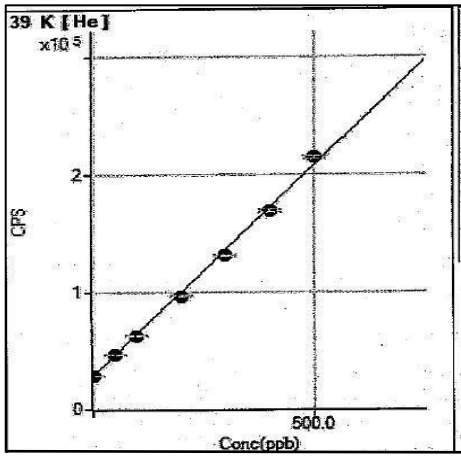
Preparation of Sample Blank Solution:

Sample blank solution was prepared without sample and followed same procedure, described under procedure of sample solution preparation. The prepared samples were kept into microwave digestion vessel for 1900 for 2 minutes. The vessel was cooled down and the pressure of the sample was maintained. The sample information and calibration curve information was entered into ICPMS software. The analysis of the sample was done, followed by the calibration levels. Then calibration graph and final value of elements were detected through ICPMS software in the leaves samples of E. microphylla.

RESULTS

The calibration curve of various elements like Iron, Potassium, Calcium, Zinc**,** Manganese, Copper and Chromium, detected in E. microphylla.by the help of ICP-MS are illustrated below in figures (4- 10)**.**

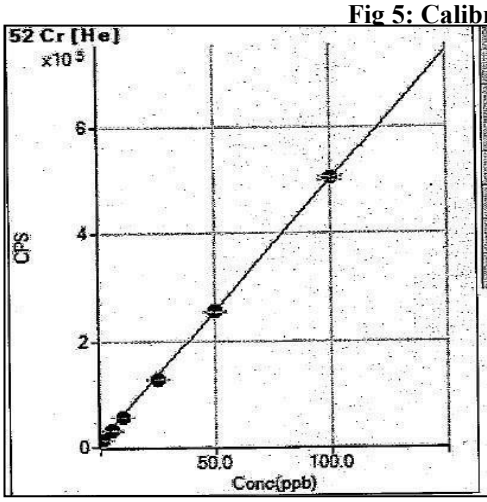
Fig 4: Calibration graph of Potassium



	Rjct	Conc	Calc Conc	CPS	Ratio	Det	RSD
1		0.000	0.000	28563.80		P	1.8
2		50.000	49.471	46260.13		P	0.7
3		100.000	96.034	62916.68		P	0.6
4		200.000	189.830	96468.91		P	1.1
5		300.000	287.137	131277.08		P	0.6
6		400.000	392.545	168983.07		P	1.7
7		500.000	518.595	214072.91		P	1.2

y = 357.7147 * x + 28563.8033
R = 0.9987
DL = 4.25
BEC = 79.85
Weight: <None>
Min Conc: 0

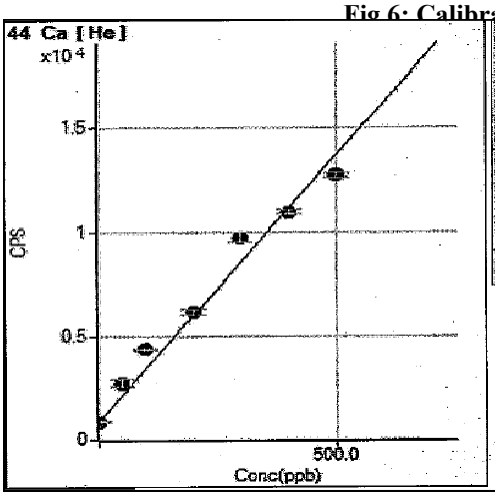
Fig 5: Calibration graph of Chromium



	Rjct	Conc	Calc Conc	CPS	Ratio	Det	RSD
1		0.000	0.000	12452.85		P	1.5
2		1.000	-0.005	12429.35		P	0.8
3		5.000	3.760	30764.71		P	3.8
4		10.000	8.696	54801.96		P	0.6
5		25.000	23.864	128667.69		P	0.7
6		50.000	49.793	254945.71		P	0.8
7		100.000	100.590	502323.02		P	1.3

y = 4869.9765 * x + 12452.8500
R = 0.9999
DL = 0.1162
BEC = 2.557
Weight: <None>
Min Conc: 0

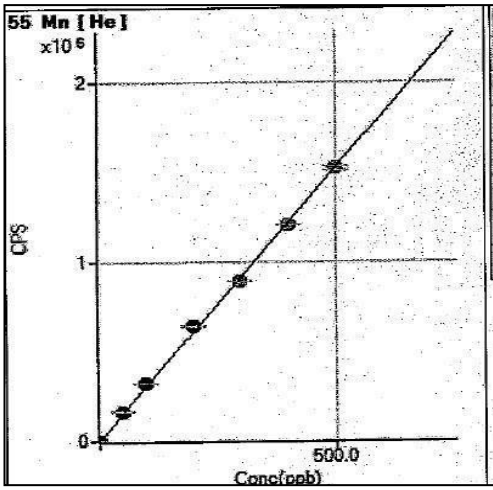
Fig 6: Calibration graph of Calcium



	Rjct	Conc	Calc Conc	CPS	Ratio	Det	RSD
1		0.000	0.000	830.97		P	9.9
2		50.000	73.975	2726.63		P	10.6
3		100.000	137.279	4348.86		P	2.4
4		200.000	207.233	6141.51		P	5.0
5		300.000	347.039	9724.15		P	4.8
6		400.000	394.348	10936.48		P	2.1
7		500.000	463.551	12709.88		P	1.9

y = 25.6259 * x + 830.9667
R = 0.9902
DL = 9.593
BEC = 32.43
Weight: <None>
Min Conc: 0

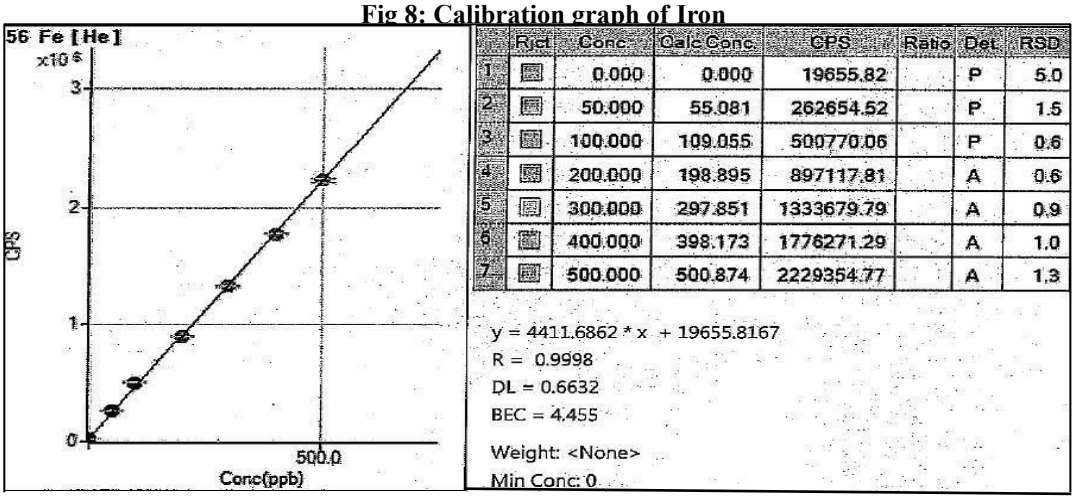
Fig 7: Calibration graph of Manganese



	Rjct	Conc	Calc Conc	CPS	Ratio	Det	RSD
1		0.000	0.000	363.75		P	3.2
2		50.000	53.755	163953.10		P	2.4
3		100.000	106.579	324710.82		P	0.5
4		200.000	211.044	642624.10		P	1.1
5		300.000	294.459	896477.27		A	0.3
6		400.000	396.693	1207602.67		A	0.0
7		500.000	499.861	1521568.36		A	1.4

y = 3043.2546 * x + 363.7500
R = 0.9996
DL = 0.0114
BEC = 0.1195
Weight: <None>
Min Conc: 0

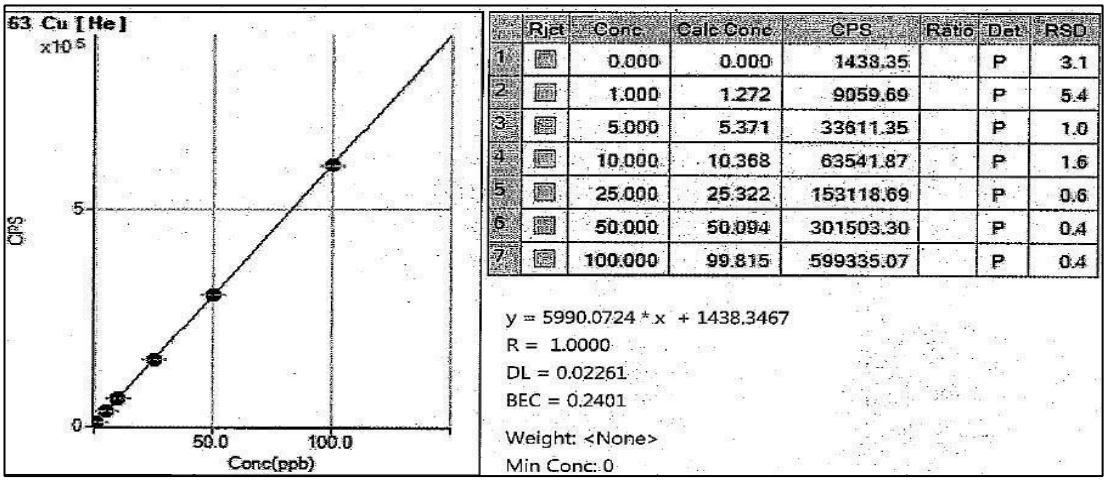
Fig 8: Calibration graph of Iron



	Rjct	Conc	Calc Conc	CPS	Ratio	Det	RSD
1		0.000	0.000	19655.82		P	5.0
2		50.000	55.081	262654.52		P	1.5
3		100.000	109.055	500770.06		P	0.6
4		200.000	198.895	897117.81		A	0.6
5		300.000	297.851	1333679.79		A	0.9
6		400.000	398.173	1776271.29		A	1.0
7		500.000	500.874	2229354.77		A	1.3

y = 4411.6862 * x + 19655.8167
R = 0.9998
DL = 0.6632
BEC = 4.455
Weight: <None>
Min Conc: 0

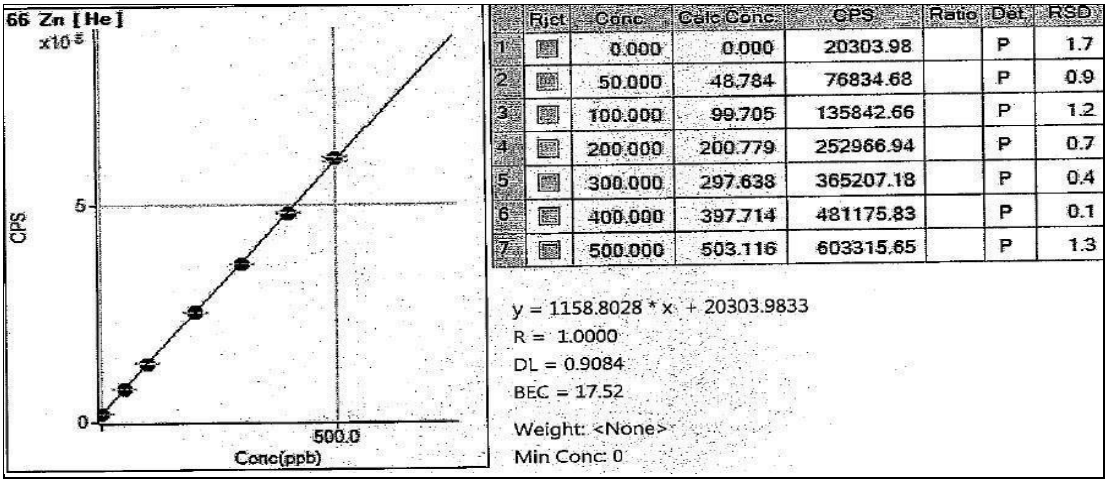
Fig 9: Calibration graph of Copper



	Rjct	Conc	Calc Conc	CPS	Ratio	Det	RSD
1		0.000	0.000	1438.35		P	3.1
2		1.000	1.272	9059.69		P	5.4
3		5.000	5.371	33611.35		P	1.0
4		10.000	10.368	63541.87		P	1.6
5		25.000	25.322	153118.69		P	0.6
6		50.000	50.094	301503.30		P	0.4
7		100.000	99.815	599335.07		P	0.4

y = 5990.0724 * x + 1438.3467
R = 1.0000
DL = 0.02261
BEC = 0.2401
Weight: <None>
Min Conc: 0

Fig 10: Calibration graph of Zinc



	Rjct	Conc	Calc Conc	CPS	Ratio	Det	RSD
1		0.000	0.000	20303.98		P	1.7
2		50.000	48.784	76834.68		P	0.9
3		100.000	99.705	135842.66		P	1.2
4		200.000	200.779	252966.94		P	0.7
5		300.000	297.638	365207.18		P	0.4
6		400.000	397.714	481175.83		P	0.1
7		500.000	503.116	603315.65		P	1.3

y = 1158.8028 * x + 20303.9833
R = 1.0000
DL = 0.9084
BEC = 17.52
Weight: <None>
Min Conc: 0

The calibration curve of various elements, detected in Ehretia Manganese were detected in the medium range of 5.41 to microphylla by ICP-MS, demonstrates the quantity of metal 35321.66 ppm sample concentration of Ehretia Microphylla. which is represented above in Figs.410. The concentrations of The above graphs represent the straight line equation y = mx +c, Iron, Calcium, Potassium,

Copper, Chromium, Zinc and with slope value c and intercept-y. The variables x and y relate to coordinates x & y on the line. When we put the value for x as it is determined by the value of x. The iron is detected in the into the equation y = mx + c, we get a result for y. This means concentration range of 236.72 ppm and manganese is found in that x is an independent variable, and y is a dependent variable 136.29 ppm concentration.

Table 5. Percentage of Standard Check before the recovery of metals in the leaves of Ehretia microphylla.

Content Calculation				**% of Standard Check before metal recovery**		
Name of elements	**Sample in blank ppb**	**Sample conc.in ppb**	**Result in ppm**	**Actual conc. In ppb**	**Observed conc. in ppb**	**% Recovery**
Potassium(K)	0	114.254	5597.94	400	394.4	99
Calcium (Ca)	0	144.183	35321.66	400	387.961	97
Chromium(Cr)	0.409	11.458	5.41	50	49.353	99
Manganese(Mn)	0.058	278.219	136.29	400	400.967	100
Iron (Fe)	0	483.153	236.72	400	399.75	100
Copper(Cu)	0	31.2	15.29	50	49.686	99
Zinc (Zn)	0	58.416	28.62	400	399.629	100

Table 6.Percentage of Standard Check after the recovery of metals in the leaves of Ehretia microphylla.

Content calculation				**% of Standard Check after metal recovery**		
Name of elements	**Sample blank ppb in**	**Sample conc.in ppb**	**Result in ppm**	**Actual conc. ppb in**	**Observed conc in ppb**	**% Recovery**
Potassium (K)				400	409.498	102
Calcium (Ca)				00	422.493	10
Chromium (Cr)				50	50.8	102
Manganese (Mn)				400	408.211	102
Iron (Fe)				400	409.257	102
Copper(Cu)				50	50.409	101

Zinc (Zn)			400	402.929	101

The content calculation of each metal was performed by the help of following formula-

Content calculation in ppm:	(Sample Conc. -Sample Blank Conc.)x Made up volume / Sample Wt × 1000

Calcium is found in concentration range of 35321.66 ppm, Chromium is found at 5.41 ppm whereas Copper at 15.29 ppm concentration range. The percentage of standard check before and after the recovery of metals like potassium, calcium, zinc, copper, chromium, manganese etc in the leaves of Ehretia The metals detected in Ehretia microphylla by ICPMS, are reported to have many physiological and medicinal importance.

Iron (Fe)

Iron is an important element for the functioning of human metabolism, it is present in Myoglobin, Haemoglobin, and other important enzymes for carrying oxygen to blood. It plays an important role in energy production and strong immunity[8]. The Iron concentration in Ehretia microphylla analyzed was 236.72ppm which is a very good amount to use for medicinal purposes.

Copper (Cu)

Copper is the most important element in the body after zinc and iron. It is an important element to carry iron in the blood. Its deficiency causes cardiovascular diseases. Ehretia microphylla leaf extract taken from the sample was analyzed and found to contain 15.29 ppm copper[9].

Chromium (Cr)

The chromium concentration in the Ehretia microphylla leaf extract of the analyzed sample was 5.41 ppm. Chromium concentration affects lipid, protein, and carbohydrate metabolism. The high content can therefore be attributed to the use of volatile insecticides and above all the beneficial copper oxide[11].

Manganese (Mn)

Manganese plays an important role in calcium absorption, fat and carbohydrate metabolism, blood clotting etc[12]. The manganese concentration in the Ehretia microphylla leaf extract of the analyzed sample was 136.29 ppm.

Calcium (Ca)

Calcium has several important functions, including helping with bone formation and keeping teeth healthy. It regulates muscle contractions, including hear rate, and ensures normal blood clots[16]. The calcium concentration in the Ehretia microphylla leaf extract of the analyzed sample was found to be 35321.66 ppm.

microphylla is depicted in tables 5 & 6, which represent the concentration of various metals in ppb, their content calculation, actual concentration and observed concentration of metal and their percentage recovery.

Potassium (K)

Potassium helps to maintain the body fluid inside the cells, contraction of muscle, and blood pressure. Analysis of a sample of Ehretia microphylla leaf extract revealed a potassium content of 5597.94 ppm which is an appreciable amount for medicinal purposes.

Zinc (Zn)

Zinc is considered as one of the main components of over 200 enzymes having both catalytic and structural roles including alcohol dehydrogenase, ribonucleic polymerases, alkaline phosphatase, and carbonic anhydrase. Scientific studies conducted on animals have shown that zinc deficiency occurred during pregnancy and may cause developmental disorders in the offspring. Low intake of zinc may cause coronary artery disease. The concentration of zinc in Ehretia microphylla leaf extracts of the sample was analyzed and found to be 28.62 ppm. Final concentrations of various metals detected in leaf extracts of Ehretia microphylla is given in the following table 7.

Village, Bangladesh. The Science of The Total Environment,

Table 7: Metal detection (concentration in ppm) in leaf extracts of Ehretia microphylla by ICP- MS

Elements content Identified by using ICP-MS	TEST METHOD / STANDARD	Concentration in ppm
CALCIUM	NSL-MOA-PH-0018-02	35321.66 ppm
MANGANESE	NSL-MOA-PH-0018-02	136.29 ppm
IRON	NSL-MOA-PH-0018-02	236.72 ppm
CHROMIUM	NSL-MOA-PH-0018-02	5.41 ppm
COPPER	NSL-MOA-PH-0018-02	15.29 ppm
POTASSIUM	NSL-MOA-PH-0018-02	5597.94 ppm
ZINC	NSL-MOA-PH-0018-02	28.62ppm

Thus in leaves extract of Ehretia microphylla, quantity of metals like manganese, iron, chromium, copper, Potassium, Zinc and calcium are found at 136.29ppm, 236.72 ppm, 5.41 ppm,15.29ppm, 5597.94 ppm,28.62ppm and 35321.66 ppm respectively as detected by ICPMS.

CONCLUSION

The present study reveals that Ca, Cu, Mn, Cr, K, Fe and Zn metals are present in Ehretia microphylla. The tested plants showed that the presence of Ca, and K might be a useful supplement because these two elements are essential elements in trace concentrations for body metabolism. Hence, the plant can be used for the development of drugs and herbal products. Thus, on the basis of experimental outcome, it can be accomplished that the plant Ehretia microphylla which has vast ethano-medicinal values estimated is safe and may not produce any harmful effect of metal toxicity during their remedial application and could also develop new drug entities from the plant.

ACKNOWLEDGEMENT

The authors express their acknowledgement towards the Director and Lab technicians of Neo Science Laboratory, Chennai. The authors also extend their gratitude towards Mr. Jeetesh Kumar Sain for his support and assistance during the research.

REFERENCES

Alam, M. G. M., Snow, E. T., & Tanaka, A. (2003). Arsenic and heavy metal contamination of vegetables grown in Samta 308, 83–96.

Belay K, Tadesse A, Kebede T. Validation of a method for determining heavy metals in some ethiopian spices by dry ashing using atomic absorption spectroscopy. Int J Innov Appl Stud. 2014;5(4):327–2.

Bianchi, G. (2003). Lipids and phenols in table olives. European Journal of Lipid Science and Technology, 105, 229–242. Blunden, S., & Wallace, T. (2003). Tin in canned food: a review and understanding of occurrence and effect. Food and Chemical Toxicology, 41, 1651–1662.

Daiane D, do Nascimento PC, Jost CL, Denise B, de Carvalho LM, Andrea K. Voltammetric determination of low molecular-weight sulphur compounds in hydrothermal vent fluids - studies with hydrogen sulfide, methanethiol, ethanethiol and propanethiol. Electro anal. 2010;22(10):1066–71.

Khan KY, Khan MA, Niamat R, Munir M, Fazal H, Mazari P. et al. Element content analysis of plants of genus Ficus using atomic absorption spectrometer. Afr J Pharm Pharmaco. 2011;5(3):317–21.

Kierdorf U, Stoffels D, Kierdorf H. Element concentrations and element ratios in antler and pedicle bone of yearling red deer (cervus elaphus) stags-a quantitative x-ray fluorescence study. Biol Trace Elem Res. 2014;162(1-3):124–33. Liu F, Liu WX, Ding WH, Lv GY, Zhou XY. Trace elements analysis by ICP-OES after microwave digestion of Medicago sativa L. seeds from different locations in Xinjiang. China Asian J Chem. 2014;26(12):3522–6.

Nergiz, C., &Engez, Y. (2000). Compositional variation of olive fruit during ripening. Food Chemistry, 60, 55–59.

Nikkarinen, M., &Mertanen, E. (2004). Impact of geological origin on trace element composition of edible mushrooms. Journal of Food Composition and Analyses, 17, 301–310. Njinga RL, Moyo MN, Abdulmaliq SY. Analysis of essential elements for plants growth using instrumental neutron activation analysis. Int J Agr. 2013:1-9.

Okigbo RN, Eme UE, Ogbogu S. Biodiversity and conservation of medicinal and aromatic plants in Africa. Biotechnol. Molecular Biology Review. 2008; 3(6):127- 134.

Perez-Trujillo, J. P., Barbaste, M., & Medina, B. (2002). Contents of trace and ultra trace elements in wines from the Canaryislands (Spain) as determined by ICP-MS. Journal of Wine Research, 13, 243–256.

Roychowdhury, T., Tokunage, H., & Ando, M. (2003). Survey of arsenic and other heavy metals in food composites and drinking water and estimation of dietary intake by the villages from an arsenic affected area of the west Bengal, India. The Science of The Total Environment, 308, 115– 135.

V Aarthi Biodiversity and conservation of medicinal and aromatic plants in Africa.

Biotechnol. Molecular Biology2012;121(3):887–92

Wang HW, Liu YQ. Evaluation of trace and toxic element concentrations in Paris polyphylla from China with empirical and chemometric approaches. Food Chem. 2010;121(3):887–92.