Analysis of trace metal through modern techniques and Anti-microbial Activity by well diffusion method of *Nepeta hindostana* and *Ricinus communis.*

Mirza Azim Beg, Rahisuddin and Ragib Ali

Himalaya Wellness Company, Faridabad, Hariyana, India

*Email: azim_0088@yahoo.com

DOI 10.51129/ujpah-2026-40-1(10)

Received –June 6, 2026
Revised – June 8, 2026
Accepted –June 12, 2026
Published – June 20, 2026

Abstract- Nowadays, the focus has been shifted towards the development of non-antibiotic substances such as plant or plant derived Phytochemicals molecules. The principle phytochemical constituents of medicinal plants have shown good fighting potential against drug resistant pathogens. Scientifically these phytochemical constituents have been proven extremely potent antimicrobial agents in comparison to antibiotics. In this research article we have studied about phytochemical screening, heavy metal analysis and antimicrobial activity. The result shows that Both *N. hindostana* and *R. communis* possess significant antimicrobial properties, supporting their role as viable alternatives to synthetic antibiotics. The heavy metals in both herbs are within the prescribed limit as per WHO. Phytochemical qualitative screening show that both herbs contain essential phytochemicals responsible for their antimicrobial and antioxidant activity and make them more suitable herbs for making herbal preparation.

Key words: Heavy metal, ICP-MS, Digester, Anti-microbial activity, Antibiotics.

Introduction

Natural plants have always been valued as a mode of treatment of variety of disease in folk cultures from ancient times and have played a very important role in discovering the western medicines. Although the efficacy of medicinal plants for curative purposes is often accounted for in terms of their phytochemical constituents like tannins, bitters, flavonoids, terpenoids, saponins, essential oils, vitamins, glycolsides, etc. Now, study shows that the over dose or prolonged ingestion of medicinal plants leads to the chronic accumulation of different trace elements which causes various health problems. So, it is mandatory to evaluate elemental contents of the medicinal plants and their preparation to control their quality**.** In recent years, several authors like Wong et al., 1993 in China; Sharma et al., 2009 in India; Sheded et al., 2006 in Egypt; Koe and Sari, 2009, Basgel and Erdemoglu, 2006 in Turkey; Ajasa et al., 2004 in Nigeria; Kanias and Loukis, 1987 in Greece and many more all across the world, reported many studies on the importance of elemental constituents of the herbal drug plants which enhanced the awareness about

trace elements in these plants Most of these studies concluded that essential metals can also produce toxic effects when the metal intake is in high concentrations, whereas non-essential metals are toxic even in very low concentrations for human health. Herbal medicines are more common and most uses for treatment of diseases in India. Overseas reports are scanty with respect to elemental constituents of endemic herbal plants of India. The present study was carried out in Himalaya Wellness Company, Faridabad Haryana. Rapid Industrialization and urbanization, are threat to the medicinal flora in context of heavy metals Therefore, it is important to have a look on good quality control of medicinal herbs in order to protect consumers from contamination. In recent years, considerable efforts have been made to control the spread of pathogens with various strategies, including the use of alternative anti-microbial compounds. As the pipeline of development for newer antibiotics look bleak, therefore the focus has been shifted towards the development of non-antibiotic substances such as plant or plant-derived Phytochemicals. The principle phytochemical constituents of medicinal plants have shown good fighting potential against drug resistant pathogens. Scientifically these phytochemical constituents have been proven extremely potent antimicrobial agents in comparison to antibiotics. Due to their antimicrobial activity, these natural ingredients appear as a viable and healthy alternative to synthetic antibiotics for the treatment of different diseases. Chemical analysis of medicinal plants revealed the presence of several ingredients, most of which possess important antioxidant and anti-microbial properties. The primary aim of this study is to establish the trace metals like Pb, Cd, As, Li & Hg levels two herbs *Nepeta hindostana* and *Ricinus communis* by ICP-MS instrument along with Antimicrobial study of the same herbs against *Escherichia coli* (ATCC-8739), *Salmonella* (NCTC6017), *Pseudomonas aeruginosa*(ATCC-9027), *Staphylococcus aureus* (ATCC-6538), *Candida albicans* (ATCC-10231)[1-15].

Material and Methods

Sample collection- The plant materials were collected from the approved vendors of Himalaya Wellness Company, Faridabad, Haryana.

Sample preparation- Plant samples were washed with deionized water and oven dried at 40°C for 2 days and then subjected to grinding for powder formation.

Phytochemical screening

Steroids- Three ml of test solution and minimum quantity of chloroform was added with 3-4 drops of acetic anhydride and one drop of concentrated H2SO4. Purple color thus formed changes into blue or green color indicating the presence of steroids.

Triterpenoids- A 3 ml of test solution was added with a piece of tin and 2 drops of thionyl chloride. Formation of violet or purple colour indicates the presence of triterpenoids.

Carbohydrate (Molisch's Test)- Add a few drops of alcoholic α-naphthol to the plant extract, followed by concentrated sulfuric acid (H_2SO_4) carefully poured down the side of the test tube. A purple or reddish-violet ring at the junction of the two liquids indicates the presence of carbohydrates.

Alkaloids- A 3 ml of test solution was taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent. Formation of white precipitate or turbidity indicates the presence of alkaloids.

Phenols- A 3 ml of test solution in alcohol was added with one drop of neutral ferric chloride (5%) solution. Formation of intense blue color indicates the presence of phenols.

Flavonoids- A 3 ml of test solution in alcohol was added with a bit of magnesium and one (or) two drops of concentrated HCl and heated. Formation of red or orange color indicates the presence of flavonoids.

Saponins- A 3 ml of test solution was foamy lather indicates the presence of Saponins.

Tannins- A 3 ml of test solution was added with water and lead acetate. Formation of white precipitate indicates the presence of tannins.

Amino Acids- A 3 ml of test solution was added with 1% ninhydrin in alcohol. Formation of blue or violet color indicates the presence of amino acids.

Digestion of sample for trace metal analysis-Weigh about 250 mg powder of each plant sample into vessel and add 0.5 ml of gold solution 10 ppm, add 8 ml trace metal Nitric acid, swirl the vessel content and allowed to keep for 5 minutes to complete any reaction, seal the vessel which are then put into cavity of digester in a manner prescribed by manufacturer. Set the method by clicking on touch screen of microwave digester, the common points are temperature ramp and temperature hold as we worked on Anton Paar microwave digester model number Multiwave 3001 50 HZ. This model is temperature control and we have set temperature ramp of 180 ° C for 20 minutes and temperature hold of 180 °C for 20 minutes. After completion of digestion cycle, remove the sample from the cavity and filter through whatman filter paper 1 into 50 ml plastic tubes and make up the volume with Milli Q water, care should be taken that all the digested sample should be clear free from any residue. The filtered sample is ready for ICP-MS analysis.

Standard preparation

1. **Diluent-**2% Trace metal Nitric Acid solution in Milli Q water.
2. **Gold solution (10 ppm)-** transfer 0.5 ml of gold standard stock sol (1000 ppm) into 50 ml plastic tube and makeup the volume with diluent.
3. **Stock Standard solution A (Mercury 10 ppm):** Transfer 0.5 ml of 1000 ppm Mercury standard solution and 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube, makeup the volume with diluent.
4. **Stock Standard solution B (Mercury 100 ppb, multi element 1000 ppb :** Transfer 0.5 ml of 100 ppm Multi element standard solution, 0.5 ml of standard stock solution A and 0.5 ml of gold solution 10 ppm into 50 ml plastic tube, makeup the volume with diluent.

Working Standard solution:

1. **Standard Blank:** transfer 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube and makeup the volume with diluent.

2. **Standard 1 (Mercury 0.1 ppb, Multi element 1 ppb):** transfer 0.05 ml standard stock solution B and 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube and makeup the volume with diluent.
3. **Standard 2 (Mercury 0.5 ppb, Multi element 5 ppb):** transfer 0.25 ml standard stock solution B and 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube and makeup the volume with diluent.
4. **Standard 3 (Mercury 1 ppb, Multi element 10 ppb):** transfer 0.5 ml standard stock solution B and 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube and makeup the volume with diluent.
5. **Standard 4 (Mercury 2.5 ppb, Multi element 25 ppb):** transfer 1.25 ml standard stock solution B and 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube and makeup the volume with diluent.
6. **Standard 5 (Mercury 5 ppb, Multi element 50 ppb):** transfer 2.5 ml standard stock solution B and 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube and makeup the volume with diluent.
7. **Standard 6 (Mercury 7.5 ppb, Multi element 75 ppb):** transfer 3.75 ml standard stock solution B and 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube and makeup the volume with diluent.
8. **Standard 7 (Mercury 10 ppb, Multi element 100 ppb):** transfer 5 ml standard stock solution B and 0.5 ml of gold solution of 10 ppm into 50 ml plastic tube and makeup the vol. with diluent.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)- is a highly sensitive analytical technique used to detect and quantify trace-to-ultratrace elements (metals and non-metals) down to parts-per-trillion (ppt) levels. It operates by ionizing samples in a high-temperature argon plasma and measuring the ions using a mass spectrometer. For this study we use Perkin Elmer NexION 1100 model. The parameters we have select for the analysis are: Sweeps/Reading 40, Repli-cates3, Peak Hoping, Standard mode, Dwell time per AMU 50, Integration time 2000, Mass 207.977 for Pb, 110. 904 for Cadmium, 74.9216 for As, 201. 971 for Hg and 7.016 for Li. Curve type is Linear through zero, peristaltic pump speed for sample flush -48 rpm and time is 75 seconds, read delay speed -20 rpm for 15 minutes and for washing speed -48rpm and time is 75 seconds. Reporting is select as Quant Summary+ QC rop.

Culture medium and inoculum preparation-The test organisms were sub-cultured onto fresh plates of Soybean Casein Digest Agar (Hi Media laboratories) and Sabouraud Dextrose Agar (Hi Media laboratories) for 24 hours at 37 ℃ and 25-30 ℃ for 3-5 days respectively. Colonies from these plates were suspended in Soybean Casein Digest Broth and Sabouraud Dextrose Broth. The media used for antimicrobial assays were Soybean Casein Digest Agar for bacteria and Sabouraud Dextrose Agar for fungi. All were incubated appropriately as specified for each organism for a period of 18–24 h (Burt 2004).

Antimicrobial Study

Agar Well Diffusion Method- The antimicrobial activities of the selected *Nepeta hindostana and Ricinus communis methanolic aquous and Acetone* by Agar well diffusion method. In this method, 100µl of standardized inoculum of each test bacterium were spread onto sterile Soybean Casein Digest Agar and Sabouraud Dextrose Agar for bacteria and fungus respectively. 6 mm diameter well was cut from the agar using a sterile cork-borer; subsequently each well was filled with 100 µl of *Nepeta hindostana and Ricinus communis*. The plates were kept at room temperature for 1 h to allow proper diffusion of the plant extracts into agar and then incubated at 37℃ for 24 h and 25-30℃ for 3–5 days respectively. Triplicates were prepared for each sample. The plant extracts having antimicrobial activity inhibit the microbial growth and the clear zones were formed. The zone of inhibition was measured in millimeters. The percentage activities of essential oils were calculated against standard drugs which was considered as higher.

Strains of tested organisms- The bacterial strains used in the study were obtained from the Microbiology Lab., Department of Quality Control and Quality Assurance Himalaya Wellness Company Faridabad Haryana. *Escherichia coli* (ATCC-8739), *Salmonella* (NCTC6017) *Pseudomonas aeruginosa* (ATCC-9027), *Staphylococcus aureus* (ATCC-6538) and *Candida albicans* (ATCC-10231) use for this study[16-26].

Results and Discussion

Table-1 Qualitative phytochemical constituents of *Nepeta hindostana and Ricinus communis* **in methanolic extract.**

Phytochemical Constituents	***Nepeta hindostana***	***Ricinus communis***
Carbohydrates	-	+
Protein	-	+
Alkaloids	-	+
Steroids	-	-
Phenolic compound	+	+
Terpenoids	-	+
Flavonoids	+	-
Saponins	-	-
Tannins	-	+

+ = Present, - = Absent

Table-2 Concentration of trace metals in *Nepeta hindostana and Ricinus communis*

Sample Name	Lead (pb)	Cadmium (cd)	Arsenic (As)	Mercury (Hg)	Lithium (Li)
Nepeta hindostana	0.849 ppm	0.072 ppm	0.322 ppm	0.010 ppm	1.264 ppm
Ricinus communis	1.137 ppm	0.076 ppm	0.107 ppm	0.012 ppm	0.281 ppm

Table-3 Antimicrobial activity of the Methanolic, Aqueous and Acetone extracts of selected medicinal plants

Sr. No	Test Microorganisms	Zone of Inhibition (mm)							
		Nepeta hindostana			*Ricinus communis*			Positive Control	
	Microbial Strains	100 µL Meth.	100 µL Aqu.	100 µL Acet.	100 µL Meth.	100 µL Aqu.	100 µL Acet.	100 µL Amox.	100 µL Itrac.
1	*Escherichia coli* (ATCC-8739)	20	18	14	17	14	12	26	-
2	*Salmonella* (NCTC6017)	18	16	12	16	14	11	26	-
3	*Pseudomonas aeruginosa* (ATCC-9027)	17	15	13	15	13	11	27	-
4	*Staphylococcus aureus* (ATCC-6538)	23	20	16	20	18	14	28	-
5	*Candida albicans* (ATCC-10231)	20	17	15	18	17	13	-	26

Using antibiotic: Bacteria – Amoxicillin (10mcg/ml); Fungi – Itraconazole (10mcg/ml),
Meth. = Methanol, Aqu. =aqueous and Acet. = Acetone

Discussion

Phytochemical analysis revealed the presence of phenols and flavonoids in N. hindostana, while R. communis tested positive for carbohydrates, proteins, alkaloids, phenols, terpenoids, and tannins. ICP-MS results showed that trace metal concentrations were within the limits as per WHO, with R. communis exhibiting higher Lead (1.137 ppm) and N. hindostana showing higher Lithium (1.264 ppm) levels. In anti-microbial assays, the methanolic extracts of both plants demonstrated the highest potency. N. hindostana showed superior inhibitory activity, particularly against S.aureus (23 mm zone of inhibition) and E. coli (20 mm), compared to R. communis.

Conclusion

Both *N. hindostana* and *R. communis* possess significant antimicrobial properties, supporting their role as viable alternatives to synthetic antibiotics. The study highlights the importance of ICP-MS in quality control to ensure that elemental concentrations remain within safe limits for human consumption, protecting consumers from potential heavy metal toxicity.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

References

1. Chan, K. Some aspects of toxic contamination in herbal medicines. *Chemosphere*; 2003; 52:1361-1371.
2. Haider, S.; Naithani, V.; Barthwal, J. and Kakkar, P. Heavy metal content in therapeutically important medicinal plants. *Bull. Environmental Contam. Toxicol.*; 2004; 72: 119-127.

3. Devi, N.K.; Sharma, N.H. and Kumar, S. Estimation of essential and trace elements in some medicinal plants by PIXE and PIGE techniques. *Nucl. Instrum. Meth. Phys. Res. B*.; 2008; 266: 1605-1610.
4. Sharma, K.R.; Agrawal, M. and Marshall, M.F. Heavy metals in vegetables collected from production and market sites of a tropical urban area of India. *Food Chem. Toxicol*.; 2009; 47:583-591.
5. Somer, E. The toxic potential of trace metals in foods: A review. *J. Food Sci.*; 1983; 39: 215-217.
6. Schroeder, H. Influence of Cr, Cd and Pb on rat aortic lipids and circulating cholesterol. *Am. J. Physiol.*; 1965; 209: 433.
7. Liang, J.; Wang, Q.Q. and Huang, B.L. Concentration of hazardous heavy metals in environmental samples collected in Xiaman, China as determined by vapor generation non-dispersive atomic fluorescence spectrometry. *Anal. Sci*.; 2004; 20: 85
8. Arceusz, A.; Radecka, I. and Wesolowski, M. Identification of diversity in elements content in medicinal plants belonging to different plant families. *Food Chem.*; 2010; 120: 52-58.
9. Wong, M.K.; Tan, P. and Wee, Y.C. Heavy metals in some of Chinese herbal plants. *Biol. Trace Elem. Res.;* 1993; 36: 135-142.
10. Sheded, G.M.; Pulford, I.D. and Hamed; I.A. Presence of major and trace elements in seven medicinal plants growing in the South-Eastern Desert. *Egypt. J. Arid Env.*; 2006; 66: 210-217.
11. Koe, H. and Sari, H. Trace metal contents of some medicinal, aromatic plants and soil samples in the Mediterranean region, Turkey. *J. Appl. Chem. Res.*; 2009; 8: 52-57.
12. Basgel, S. and Erdemoglu, S.B. Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey. *Sci. Total Env.*; 2006; 359: 82-89.
13. Ajasa, M.A.; Bello, O.M.; Ibrahim, O.M.; Ogunwande, A.I. and Olawore, O.N. Heavy trace metals and macronutrients status in herbal plats of Nigeria. *Food Chem.;* 2004; 85: 67-71.
14. Kanias, G.D. and Loukis, A. Determination and correlation of active consitituents and trace elements in the medicinal plants *Thymus capitatus* Hffm. and Link, Frezenius Z. *Analyt. Chem*.; 2004; 327: 355-357.
15. Shazia, Jabeen; Muhammad, Tahir Shah; Sardar Khan and Muhammad Qasim Hayat. Determination of major and trace elements in ten important folk therapeutic plants of Haripur basin, Pakistan. *Journal of Medicinal Plants Research*; 4 April, 2010; Vol. 4(7):559-566
16. Raquel, B.; Cristina, N. and Rafael, G. Evaluation of bacterial resistance to essential oils and antibiotics after exposure to oregano and cinnamon essential oils. *Food Borne Pathog. Dis.;* 2012; 9:699–705
17. Sonboli, A.; Salehi, P. and Yousefzadi M. Anti-microbial activity and chemical composition of the essential oil of Nepeta crispa Willd, from Iran. Z Naturforsch C; 2004; 59:653–656.
18. Ahmad, I. and Beg, A. Z. Antimicrobial and phytochemical studies on 45 Indian

medicinal plants against multi-drug resistant human pathogens. *J Ethnopharmacol.*; 2001; 74:113-123.

19. Chavan, M.J.; Shinde, D.B. and Nirmal, S.A. Major volatile constituents of Annona squamosa L., bark. *Nat. Prod. Res.;* 2006; 20:54–757.
20. Raid, A. A.; Yazeed, A.S.; Ayesha, M.; Rabbani, S.K.; Janardhan, C. and Gupta, V.C. Evaluation of antibacterial activity of crude protein extracts from seeds of six different medical plants against standard bacterial strains. *Saudi J. Biol. Sci.;* 2014; 21:147–151.
21. Syed Amir, Ashraf; Eyad, Al-Shammari; Hussain, Talib; Tajuddin, Shaikh and Bibhu Prasad Panda. In-vitro antimicrobial activity and identification of bioactive components using GC–MS of commercially available essential oils in Saudi Arabia *J. Food Sci. Technol.;* November; 2017; 54(12):3948–3958.
22. Reeves, D.S. Antibiotic assays. In: Hawkey PM, Lewis DA (eds) Medical bacteriology, a practical approach. *IRL Press, Oxford*; 1989; pp 195–221
23. Raid, A.A.; Yazeed, A.S.; Ayesha, M.; Rabbani, S.K.; Janardhan, C. Gupta, V.C. Evaluation of antibacterial activity of crude protein extracts from seeds of six different medical plants against standard bacterial strains. *Saudi J. Biol. Sci.*; 2014; 21:147–151
24. Subramani, V. and Kmaraj, M. Trace metal concentration and antimicrobial efficacy of selective medicinal plants, southern India. *International Journal of Pharmaceutical, Chemical and Biological Sciences (IJPCBS)*; 2014; 4(1):182-187.
25. Maduakor Uzoamaka Charity; Egoh Chinaza Livia; Udoh Iniekong Philip; Okafor Edwin Nkemjika; Okonkwo Innocent Nwabueze and Maduakor Chima Jachi. Evaluation of antimicrobial activity of methanolic leaf extract of ricinus communis against some clinical isolates. *Pharmacology OnLine, Archives;* 2021; Vol. 3:428-436.
26. Abhay, K.; Pandey, Manindra Mohan; Singh, Pooja and Tripathi; N. Chemical Composition, Antioxidant and Antimicrobial Activities of the Essential Oil of Nepeta hindostana (Roth) Haines from India. *Rec. Nat. Prod.*; 2015; 9:224-233.