Original Research Article **Pathology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

SCREENING OF BLUMEA LACERA AND CYATHOCLINE PURPUREA (ASTERACEAE) FOR MINERAL PROFILES, HEAVY METALS AND PHYTOCHEMICALS

S.D.MANE*[1] AND V.B.SHIMPALE[2]

[1]Department of Agrochemicals and Pest Management, Shivaji University , Kolhapur, India.
[2]Department of Botany, The New College, Kolhapur, India.

ABSTRACT

The aim of this study was to analyze weed species for their screening for presence of essential and heavy metals and phytochemicals. Essential minerals are the metals which plays vital role in plants life cycle so they are needed for growth while heavy metals are the metals of relatively high density or of high atomic weight which causes some adverse effects also. For the extraction of heavy metals and essential minerals two weed samples were selected viz., *Blumea lacera* and *Cyathocline purpurea*. Both these weeds are common in occurrence and belongs to family Asteraceae. The quantitative analysis of metals from these plants has been done by using standard analytical techniques. Some metals for which these samples were analyzed are N, P, K, Ca, Mg, S, Na, Zn, Fe, Cu, Mn, Mo, B, Cd, Cr, Pb, Ni, As, Si, Hg. Atomic absorption spectrophotometer, flame photometer, UV-spectrophotometer and Kjeldhals apparatus were used for analyzing the samples. Entire plant sample was estimated to get the proportion of heavy metals in ppm or % unit. Both plant shows presence of moderate amount of all micronutrient and macronutrient. Magnesium metal shows higher concentration in *Blumea lacera* i.e.5.21% while in *Cyathocline purpurea* sulphur metal present in higher concentration i.e. 6.42%. Along with essential minerals and heavy metals, phytochemicals like tannin, flavonoid and alkaloid also analyzed quantitatively by using standard methods. Identical result is shown by using calibration curve which is obtained with the help of standard solutions.

KEYWORDS: ***Essential minerals, Heavy metals, Blumea lacera, Cyathocline purpurea, Phytochemicals.***

S.D.MANE*

Department of Agrochemicals and Pest Management,
Shivaji University , Kolhapur, India.

Corresponding Author

Received on: 28-08-2017
Revised and Accepted on: 11-10-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.4.b489-494

INTRODUCTION

Plants which are used for estimation of essential and heavy metals are from the Asteraceae family, locally they are used as medicinal herbs but commonly found in local region as a weed. *Blumea lacera* is described as a valuable medicinal plant in many vital systems of medicines including Ayurveda homeopathy and Yunani or unani. *Blumea lacera* is an erect villous herb.This plant is astringent, antispasmodic, stomachic, antipyretic as well as diuretic, cures bronchitis, fever, and also burning sensation. Leaf juice is anthelmentic and stimulant, mixed with pepper and it is given in treatment to cure piles. Roots mixed with pepper are given for the treatment of cholera and along with rhizomes of *Cyprus rotundus* also given in dysentery. An alcoholic extract of the herb exhibited marked anti-inflammatory activity. It shows great antimicrobial activities as well as its essential oil also shows some medicinal properties hence this weed used as medicinal plant.[1]*Cyathocline purpurea* is an annual and occasionally perennial. Flowers are usually purple in colour and occur in corymbs at the end of branches. This weed plant is also shows great importance in medicinal field mostly as an anti-inflammatory agent so it is also a medicinal plant species. *Cyathocline* species are active as a medicinally important plants.[2]The plant used in medicines, the roots are used in treating stomach pain. Some heavy metalsare analyzed from these two plant samples for the purpose of studying the presence of their quantitative factor. Heavy metals are present in varying concentration in different plants. They are found in elemental form and in a variety of other chemical compounds. Aheavy metal,depending on the context, is usually regarded as ametalor sometimes ametalloid with high density and atomic weightor withatomic number, is often assumed to be toxic metal. Some heavy metals, such as cadmium, chromium, mercury, arsenicand leadare highly toxic.[3-5]Whileothers are essential nutrients in trace amounts or are relatively harmless. Some essential mineralslike iron, zinc, copper, cobalt, sodium and manganese are needed for proper physiological function while they present in low amount; however, their higher concentrations of these metals can be toxic.[6] There are other metals such as mercury, lead that are toxic to living things.[7] Some heavy metals given below shows their presence in result of chemical analysis. There are total 20 elements were analyzed out of that 13 are essential nutrients while others are toxic heavy metals but essential nutrients also shows adverse effects on plant growth metabolism like nickel as well as some enzymatic and biochemical reactions occur within cells when they are present in excess amount.[8,9]Phytochemical means an active chemical compound which shows medicinal properties. All primary and secondary chemical compounds are included in phytochemical.Tannin is the most important phytochemical found in the plant species. It has unique properties like strange smell and taste. It has property to bind with proteins. Tannin has antihelminthic, antimicrobial, antiviral, antioxidant and anti-inflammatory properties.[10]Tannin is present in growing organs of plants because it may help to regulate the growth of plant growing tissues. Plants from Asteraceae family contain less amount of tannin and hence presence of phytochemicals including tannin it shows good results towards medicinally important activities.[11]Tannins can be classified into two types such as hydrolysable tannin and non-hydrolysable tannin or condensed tannin.[12]Most of the plants contain flavonoid as Phytochemical. Flavonoid is a water soluble phytochemical and is a phytophenolic compound which has antioxidant potential.[13]Flavonoid shows anti-inflammatory, antimicrobial, anti-tumor as well as anti-oxidant properties.[14]Alkaloid is also an important phytochemical in various plant species. It is a natural product and contains nitrogen atoms. Hence alkaloid is alkaline product which contains nitrogenous bases. It shows vital function for protection plants for their survival.[14]In the present investigation, two weed species viz. *Blumea lacera* and *Cyathocline purpurea* were assessed for their mineral composition and heavy metal profiling. Moreover, these two weeds were also evaluated for their phytochemical profiling including total alkaloids, flavonoids and tannin content.

MATERIAL AND METHODS

Plant samples were selected for investigation are *Blumea lacera* and *Cyathocline purpurea*.(Dr. V. B. Shimpale, Department of Botany, ShivajiUniversity,Kolhapur.). Plant sample collected from local area (Kolhapur region). After collecting both fresh plant samples, they get separated from dirt and adhered soil as well as followed by washing and cleaning under water flow to remove attached dust. The whole fresh clean plant samples were dried by introducing to direct sunlight. Again these air dried samples were oven dried to remove total moisture content at temperature 60-70˚C by maintaining constant weight. The dried plant materials were ground and sieved to get fine powder. These crushed sample powder get stored in two air tight containers and were used for further analysis.

Acid digestion of sample

Dried plant sample powder weighed 2.0gm and taken in crucible and ignited to 450˚C for 3hrs.in muffle furnace. After heating ash sample formed which was transferred to beaker and accurately 5ml of 6M HCl was added to it. Total moisture free ash sample mixed with acid for digestion purpose only. This acidic medium containing sample was kept on hot plate and it forms clean, clear solution which contains trace and heavy metals present in that ash sample. Finally prepared solution and residue again dissolved in 0.1 M HNO_3 to made the final volume 50ml. By the action of hydrochloric acid and nitric acid all the heavy metals get dissolved in it and forms stock solution. This stock solution is used for further analysis by making dilutions with distilled water.[15]

AAS analysis of sample

AAS analysis is a chemical technique which is used widely to analyze the sample with the help of instrument Atomic absorption spectrometer.[16]This technique works on the principle of Beer's law.[17]In this technique sample dilutions are analysed to determine the amount of metals or elements present in percentage.[18]

Flame photometric analysis

Analysis of sample using flame photometry which is based on flame emission spectroscopy. It works on the principle of Lambert- Beer's law. Main events that take takes place in the analysis are vaporization, atomization, and excitation.[19]

UV-spectrophotometric analysis

UV-spectrophotometry is based on absorption, transmission of light and its reflectivity. Electrons of metals reach to higher energy level which is known to be excited state and from that they suddenly drop down to lowest energy level which is ground state.[20]

Nitrogen analysis by using kjeldahl apparatus

Determination of nitrogen content from organic as well as inorganic sample followed by three steps mainly digestion, distillation and titration is called kjeldahl's method.[21]

Determination of total moisture content and ash

For determination of total ash forming capacity of material, muffle furnace has been used. For its analysis crushed sample is weighed accurately as 1.0gm and after weighing it was introduced to muffle furnace and ignited to 450˚C for 3hrs. After ignition ash was formed and from weighing total ash percentage also determined.

Determination of phytochemicals

Alkaloid determination using Harborne (1973) method

5g of the sample was weighed into a 250 ml beaker. 200 ml of 10% acetic acid in ethanol was added to that beaker,covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Conc. Ammonium hydroxide was added drop wise to the extract, until the precipitation was done. The whole solution was allowed to settle and the precipitate was collected. Again it is washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid and was dried and weighed.

Determination of flavonoid by Aluminium chloride method

0.5 ml of extract was mixed with 1.5 ml of methanol to this mixture 0.1 ml 10% $AlCl_3$ was added. 0.1ml of 1M potassium acetate and 2.8 ml of distilled water was also added to this and kept for 30 min at room temperature. The absorbance was measured at 415 nm. A calibration graph was performed with quercetin which was used as standard for flavonoid.

Determination of total tannin content

5g of plant sample was mixed with 50ml distilled water and was shaken for 30 min. then centrifuged at 5000rpm. Suspension was diluted with 100ml distilled water. 5ml diluted extract was mixed with 1ml FCR reagent and 2.5 ml of 1M saturated sodium carbonate then the whole volume of mixture was made 50ml. After incubation for 90 min at room temperature absorbance was measured at 760nm.

RESULT

Blumealacera and *Cyathocline purpurea* are the plants which show various medicinal uses against some diseases and uncomfort conditions. Some metals were analyzed by using standard methods out of which some are essential minerals while some are heavy metals.The amountof essential minerals are given in Table 1 while amount of heavy metals are given in Table 2. Amount of alkaloid content in both weed species is determined in % by using Harborne method is shown in Table 3. Total tannin and flavonoid content was measured by using UV-spectrophotometer and with respect to tannic acid and quercetin respectively. By using standard curves their concentrations can be calculated. Table 3 shows content of tannin and flavonoid in mg/gm for *Blumealacera* and *Cyathocline purpurea*. Total ash and moisture content determined from above procedure followed by heating technique by using muffle furnace and hot air oven respectively are given in Table 4.

Table 1

Essential minerals present in Blumea lacera and Cyathocline purpurea

Sr. No.	Essential minerals	*Blumea lacera*	*Cyathocline purpurea*
1.	Nitrate (%)	0.16	5.20
2.	Phosphrous (%)	0.17	0.15
3.	Potassium (%)	0.67	0.88
4.	Calcium(%)	0.54	0.75
5.	Magnesium (%)	5.21	5.13
6.	Sulphur (%)	4.99	6.42
7.	Sodium (%)	3.31	3.85
8.	Zinc (ppm)	238.18	253.16
9.	Ferrous (ppm)	1,161.24	1,201.24
10.	Copper (ppm)	98.45	82.72
11.	Mangenese(ppm)	251.56	463.91
12	Molybdenum (ppm)	0.50	0.40
13.	Boron (ppm)	11.06	2.20

Table 2
Heavy metals present in Blumea lacera and Cyathocline purpurea

Sr. No.	Metal	*Blumea lacera*	*Cyathocline purpurea*	Standard acceptable range
1.	Cadmium (ppm)	154.43	0.17	0.30
2.	Chromium (ppm)	6.59	1.83	10
3.	Lead (ppm)	6.95	10.26	10
4.	Nickel (ppm)	Less than 0.1	7.16	0.67
5.	Arsenic (ppm)	101.71	677.73	100
6.	Silicon (ppm)	4,881.92	11,152.92	_
7.	Mercury(ppm)	210.82	94.24	2.41

Phytochemical analysis

The result confirms the presence of chemical constituents which shows medicinal as well as physiological activities. The results reveal the presence of medicinally active constituents like tannins, alkaloid and flavonoid which are termed as Phytochemical.

Table 3
Alkaloid, Tannin and Flavonoid content in Blumea lacera and Cyathocline purpurea

Parameters	*Blumea lacera*	*Cyathocline purpurea*
Alkaloid	6.1%	3.7%
Tannin	42.03mg/gm	41.74mg/gm
Flavonoid	32.56mg/gm	31.18mg/gm

Table 4
Moisture and ash content in *Blumealacera* and *Cyathocline purpurea*

Parameters	*Blumea lacera*	*Cyathocline purpurea*
Moisture (%)	6.20	7.20
Ash (%)	9.70	3.30

DISCUSSION

Evaluation after extraction and analysis of two weedy plant species namely *Blumea lacera* and *Cyathocline purpurea*for their essential as well as heavy metal analysis and about 20 heavy metals are estimated from both plant samples.The methods of analysis are different because determination of metals by using analytical technique is depends on their digestion, wavelength, absorption capacity, energy levels and emission of radiation.[22]Acid digestion using hot plate method and ashing of sample done for removal of traces and unwanted particles from crushed powder form of sample. Above results in Table 2 informs us that analysis of heavy metals by using AAS in both weed species shows silicon is present in more amount (4,881.92ppm and 11,152.92ppm). In *Blumea lacera* nickel is present in very less amount(0.1ppm) and in *Cyathocline purpurea* cadmium is in low concentration(0.17ppm). AAS analysis infers that silicon is essential for plant growth and metabolism while nickelis essential but adverse effect causing heavy metal and cadmium is toxic heavy metal, both are present in less concentration, hence it does not affects on both species and their life cycle.[23-25] Analysis of essential minerals using flame photometry determines percent concentration of four minerals in both weed species which are shown in Table1.It infersthat in both species magnesium present in high concentration (5.21% and 5.13%) while sodium present in low concentration (3.31% and 3.85%). Magnesium as well as sodium essential for chlorophyll formation and photosynthesis but then also it has low amount of sodium.UV-spectrophotometer shows identical result in percent concentration of four elements which are shown in Table 1. It infers that in *Blumea lacera,* boron shows higher concentration(11.06ppm) whilephosphorus is in less concentration (0.15%). In *Cyathocline purpurea* sulphur showed high percentage (6.42%) and in this sample also phosphorus is in less percentage (0.15%). All the three elements found essential for plant growth and its nourishment. By using Kjeldahl's apparatus, nitrogen content is determined in the form of nitrate and its percent concentration is given in Table 1. Nitrogen is an essential element for plant's nourishment, it is a building blocks of protein then also *Blumea lacera* shows less content of nitrogen (0.16%) and in *Cyathocline purpurea* it is present in moderate range (5.20%). By using all above methods result are mentioned in Table 1 and Table 2 are of quantitative determination of essential minerals and heavy metals respectively. Nitrogen is most important constituent which is source of chlorophyll and protein synthesis. It plays vital role in plant metabolism hence they are known as 'basic constituent of life.' Phosphorus is an essential nutrient which plays significant role in transformation of energy by enzymes. Potassium is required to all plants for transpiration process. It plays its function mainly in cell sap in a solution form means found in dissolved or soluble state. Calcium metal present in plant cell and its more composition is found in cell wall. It promotes growth and development of roots.[9]In green plants magnesium is present as a constituent of chlorophyll. For the nourishment, growth and development of vegetative part of the plant, sulphur is the most essential element.Boron is useful metal

having efficient use in root of plants because it concerned with calcium metabolism. Manganese help to iron movement in plant vascular system. Iron regulates various types of processes occurs within the plants such as respiration, photosynthesis. Zinc has a significant role in transpiration as well as it give and take of water. Sodium is an essential nutrient which has significant role in calvin cycle of C4 plants. While some metals like cadmium, chromium, arsenic, mercury, lead are referred as toxic heavy metal which enters in environment and causes affect on growth and development of plant.[9]Moisture percentage is determined using hot air oven and ash percentage is determined using muffle furnace which is shown in Table 4.Moisture present in higher amount in *Cyathocline purpurea* (7.20%) while ash percentage is more in *Blumealacera*(9.70%).In the quantitative evaluation of alkaloid present in *Cyathoclinepurpurea*and *Blumealacera* experimental procedure shows 3.7% and 6.1% alkaloid in samples respectively which is shown in table 3. Concentration of tannin as well as flavonoid also mentioned in Table 3.It shows amount of tannin in *Blumea lacera* is 42.03mg/gm while in *Cyathocline purpurea* it is 41.74mg/gm. Amount of flavonoid in *Blumealacera* is 32.56mg/gm while in *Cyathoclinepurpurea* it is 31.18mg/gm. Further quantification should provide more estimation regarding phytochemical properties. Weed species proved as most important plant, it has greater medicinal properties and also shows presence of some active biochemical constituents i.e. phytochemicals.[26]*Blumealacera* shows anthelmintic activity because it contains tannin, flavonoid, and alkaloids.[27]Mercury is a heavy metal which has some toxic effects on plants life cycle. And it broadly affects on plant growth and also on germination of seed. As well as its accumulation in more amount causes adverse effect on food chains.[28]Nutrients are very important and useful for all plants and its growth stages during life cycle. But its more or less amount may causes adverse effect on plants life cycle. Mercury shows stronger effect and then more amount of cadmium also causes damage to plant life. But both plant species shows very little amount of mercury and cadmium within its composition hence it does not causes any harm or reverse inhibitory effects.[29]

CONCLUSION

Blumealacera and *Cyathoclinepurpurea* were evaluated for their minerals, heavy metals and phytochemicals. The results indicated that, both the species are rich source of several essential inorganic elements as well as they accumulated the higher content s of certain heavy metals. Moreover, these species served as the chief source of alkaloids, flavonoids and tannins. There is need to investigate these species for isolation, characterization and biological activities of individual phytochemicals from both the species.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Jahan K, Kundu SK and Bake Md.A. Evaluation of antimicrobial and cytotoxic activities of the mehanolic and petroleum ether extract of BlumealaceraBurm.f in Bangladesh. J PharmacognPhytochem2014; 2 (6): 104-108.
2. Joshi A, Baghel V, Pathak AK and Tailang M. Phytochemical investigation and medicinal importance of *Cyathoclinelyrata*. Int J Res Ayurveda Pharm 2010;1(2):302-5.
3. Panda SK and Choudhary S. Chromium stress in plants. Brazilian Journal Of Plant Physiology.2005;17(1):95-102.
4. Fayiga AO, Ma LQ, Cao X and Rathinasabapathi B. Effects of heavy metals on growth and arsenic accumulationin the arsenic hyperaccumulator*Pterisvittata* L. Environmental and Experimental Botany.2008;(62): 231–37
5. Sharma P and Dubey RS. Lead toxicity in plants.Brazilian journal of plant physiology.2005;17(1): 35–52.
6. Kronzucker HJ, CoskunD,SchulzeLM,Wong JR and Britto DT. Sodium as Nutrient and Toxicant. International Journal on Plant-Soil Relationships.2013;(369):1-23.
7. Oudhia P, Kolhe SS, and Tripathi RS. Allelopathic effect of *Blumealacera*L.on rice and common to Kharif weeds.Orzya.1998;35(2):175-77.
8. Chen C, Huang D, and Liu J. Functions and toxicity of Nickel in plants:Recent advances and future prospects.www.clean-journal.com.2009;37(4–5):304-13.
9. KalamkarRJ.Textbook of 'Manures and fertilizers'.The general principles of plant nutrition,second edition.1967;7-13.
10. Ukoha PO, CemalukEAC,Nnamdi OL and Madus EP. Tannins and other phytochemicals of the *Samanaeasaman* pods and their antimicrobial activities. African Journal of Pure and Applied Chemistry.2011;5(8): 237-44.
11. Audu SA, Taiwo AE and Ojuolape AR. A Study Review of Documented Phytochemistry of Vernoniaamygdalina (Family Asteraceae) as the Basis for Pharmacologic Activity of Plant Extract. Journal of Natural Sciences Research.2012;2(7):1-9.
12. Khanbabaee K and Ree TV. Tannins: Classification and Definition. National Product Report journal of The Royal Society of Chemistry.2001;(18):641–49.
13. Ramamurthy V and Sathiyadevi M. Preliminary Phytochemical Screening of Methanol Extract of Indigoferatrita Linn. Journal of Molecular Histology & Medical Physiology.2017;2(1):1-5.
14. SaxenaM,Saxena J, Nema R, Singh D and Gupta A. Phytochemistry of Medicinal Plants. Journal of Pharmacognosy and Phytochemistry.2013;1(6):168-82.
15. Theory of Sample Preparation using Acid Digestion, Pressure Digestion and Microwave Digestion(Microwave Decomposition). MW-Theorie-Probenvorbereitung-PT-en-53-0119-95-00-00-002doc.DIN EN ISO 9001-2000:1-11.
16. Prof. Subodh Kumar. Spectroscopy of Organic Compounds.Organic Chemistry.2006.

17. Basic Atomic Absorption Theory Varian Australia Pty Ltd. (A.C.N.004559540) 1997.
18. Kumar S, Singh J, Das S and Garg M. AAS Estimation of Heavy Metals And Trace Elements In Indian Herbal Cosmetic Preparations. Research journal of chemical sciences.2012;2(3):46-51.
19. Determination of Sodium and Potassium by Flame Photometry. MeditsiinilineKeemia/Medical Chemistry,LOKT.00.009, 04.09.2012.
20. Dr. Hamid H. Ultraviolet and Visible Spectrophotometry. Pharmaceutical Analysis.2007.
21. A Guide to Kjeldahl Nitrogen Determination Methods and Apparatus LABCONCO. An Industry Service Publication.
22. Kinthada PM, Naidu PVS,Muralidhar P. Biologically estimation of heavy toxic metals present in traditional medicinal plants. *Ecliptaalba*.Int J. Pharm. Biomed Sci. 2011;2(4):99-102.
23. Garcia R and Baez AP. Atomic Absorption Spectrometry (AAS). Atomic Absorption Spectroscopy.Dr. Muhammad AkhyarFarrukh (Ed.), ISBN: 978-953-307-817-5.2012.
24. Bhalerao SA, Sharma AS and Poojari AC. Toxicity of Nickel in plants. International journal of pure and applied bioscience.2015;3(2):345-55.
25. Nazar R,Iqbal N, Masood A, Khan MRI, Syeed S and Khan NA. Cadmium Toxicity in Plants and Role of Mineral Nutrients in Its Alleviation. American journal of plant sciences.2012;3:1476-89.
26. TiwariP,Saluja G, Pandey AS and Sharma N. Isolation and biological evaluation of some Novel phytoconstituents from *Blumealacera.* International journal of pharmacy and pharmaceutical sciences. 2012;4(4).
27. Pattewar AM, Dawalbaje AB, GundaleDM,Pawar PB, Kavtikwar PG, Yerawar PP, Pandharkar TM and Patawar VA.Phytochemistryical and anthelmintic studies on *Blumea lacera*. Indo global journal of pharmaceutical sciences.2012;2(4):390-96.
28. Sahadevan KK. Physiological and biochemical studies on mercury toxicity in *Vignamango*(L.) Hepper seedling.Thesis, Department of Botany, University of Calicut.2001.
29. Oancea S, Foca N, Airinei A, Effects of heavy metals on plant growth and photosynthetic activity, 2005;107-10.

Reviewers of this article

Dr. Afroz Alam

Associate Professor
Department of Bioscience & Biotechnology,
Banasthali University,
Rajasthan, India.

Jaykumar J. Chavan, Ph.D

Assistant professor in Botany, Dept. of Botany, Yashavantrao Chavan Institute of Science, Satara,
Maharashtra 415001, India.

Prof.Dr.Prapurna Chandra Rao

Assistant Proffessor, KLE University, Belgaum, Karnataka, India

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript