PLANT SCIENCE TODAY, 2021
Vol 8(2): 324–330
https://doi.org/10.14719/pst.2021.8.2.1034
ISSN 2348-1900 (online)

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RESEARCH ARTICLE

Heavy metal stress influence the andrographolide content, phytochemicals and antioxidant activity of *Andrographis paniculata*

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ARTICLE HISTORY
Received: 20 November 2020
Accepted: 19 March 2021
Published: 01 April 2021

KEYWORDS
Andrographis paniculata
Copper
Tin
Cobalt
Andrographolide
Total phenolic content
Total flavonoids content

ABSTRACT
Heavy metals (HM) are toxic components present in the earth's crust that can have a negative impact on plants as well as animals. *Andrographis paniculata* or 'King of bitters' belonging to the family Acanthaceae, is a medicinal herb traditionally used in the treatment of fever, common cold etc. In the present study, the effect of heavy metals (copper, tin and cobalt) on the andrographolide content, biochemical parameters like chlorophyll, carotenoid, protein, Total phenolic content (TPC), Total flavonoid content (TFC) and antioxidant activity in *A. paniculata* were analysed. Saplings of *A. paniculata* were treated at 50 and 100 mM concentrations, three different times at a time interval of 7 days. Andrographolide production was found to increase in copper and cobalt treated saplings when compared with the control. From the results, maximum andrographolide concentration was found in the saplings treated with 50 mM copper (8.51 mg/gm of DW) and 50 mM tin (8.10 mg/gm of DW) respectively. 50 mM cobalt treated plants have shown the highest concentration of TPC (17.21 mg/g of extract) and TFC (6.97 mg/gm of extract). Notable variations in other biochemical parameters like total chlorophyll, carotenoid content and antioxidant activities were observed in all treatments compared with the control.

Introduction

Soil contamination is a serious threat to agriculture where heavy metals (HM) are one of the major environmental pollutants that can cause toxicity within plants. The major contributors to soil contamination are Cadmium, Copper, Lead, Chromium, Manganese and Zinc. Essential HM like Copper, Zinc, Iron, Manganese and Molybdenum are an integral parts of many enzymes that have biochemical and physiological functions within plants and animals. Some HM are highly poisonous to metal-sensitive enzymes while some are considered essential micronutrients and their uptake in higher concentration can be toxic to plants (1, 2). Copper is an essential micronutrient and a transition metal involved in the normal growth of the plant. Copper at high concentration can inhibit plant growth, biomass, photosynthesis and respiration. It can reduce the chlorophyll content, root growth and shows symptoms like chlorosis and necrosis (3). Tin is one of the naturally occurring HM at an average concentration of 2 mg/kg found on the earth and has shown an elevation up to 1000 mg/kg as a result of increased anthropogenic activities (4). Cobalt is regarded as a beneficial element required for the growth of higher plants which is important for stem growth, coleoptile elongation and also in leaf disc expansion. A higher concentration of cobalt results in adverse responses in the plants (5). Biological chromatographic fingerprinting is a quality control tool for herbal samples that helps in detecting adulterations and analysing the product's quality (6). In a study conducted with 81 samples of seven herbs in the United Arab Emirates for the detection of HM metals, it was found that 29% of the samples contained high cadmium content and 64% had lead content exceeding the permissible limit set by FAO/WHO (7).

Andrographis paniculata is a medicinal plant that has been used in traditional medicines in different countries such as India and China since ancient times. Researches reveal the pharmacological activities of the plant such as anti-cancer, anti-inflammatory, anti-angiogenic, anti-malarial and anti-hyperglycemic activities. The plant possesses a range of therapeutic

To cite this article: Antony A, Nagella P. Heavy metal stress influence the andrographolide content, phytochemicals and antioxidant activity of *Andrographis paniculata*. Plant Science Today. 2021;8(2):324–330. https://doi.org/10.14719/pst.2021.8.2.1034

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activities, which are contributed by the phytochemicals present in it such as flavonoids, diterpenes, polyphenols and stigmasterols. In addition to primary metabolites, plants also produce secondary metabolites to accomplish defense against biotic and abiotic stresses. Andrographolide is the major bitter constituent present in *A. paniculata*. It is a diterpenoid that shows enormous biological activities (8-13). As there are no previous reports on the effect of heavy metals like Cu, Sn and Co on the biosynthesis of andrographolide and other biochemicals present in *A. paniculata*, the present study was aimed to assess the impact of these heavy metals (Cu, Sn and Co) on the andrographolide production, phytochemicals and antioxidant activity in *A. paniculata.*

Materials and Methods

Chemicals and reagents

Copper sulphate, Stannous chloride, Cobalt chloride, Methanol, Acetone, Biuret reagent, Folin-Ciocalteu reagent (FC), Bovine Serum Albumin (BSA), HPLC grade methanol, Sodium carbonate, Gallic acid, Sodium nitrite, Aluminium chloride, Sodium hydroxide, Quercetin, Sulphuric acid, Sodium phosphate, Ammonium molybdate, Ferrous sulphate ($FeSO_4$), Ferrozine, Ethylenediaminetetraacetic acid (EDTA), 2,2-diphenyl-1-picrylhydrazyl reagent (DPPH) are some of the chemicals used in the present study.

Heavy metal (HM) treatment in A. paniculata

Saplings of *A. paniculata* was raised in loam soil filled pots in the polyhouse of the Christ University campus for 4 months. Two different concentrations (50 and 100 mM) of each HM were given to the saplings for three different times at a time interval of 7 days. Based on the previous set of experiments, HM concentrations >100 mM was found to be lethal to plants and concentrations <50 mM did not show any significant variation. Therefore, the concentrations, 50 and 100 mM was finally chosen for the current research. Copper sulphate, stannous chloride and cobalt chloride were the HM used for the experiment and all the tests was performed using seven samples (Control, Cu 50, Cu 100, Sn 50, Sn 100, Co 50, Co 100). After the treatment, the plants were harvested and the leaves were washed with distilled water. The fresh leaves were used for analysis of chlorophyll, carotenoid and protein content. The other harvested samples were shade dried and it was ground into fine powder using a grinder and was then stored for future experiments.

Estimation of total chlorophyll and carotenoid

Total chlorophyll and carotenoid were estimated using 80% acetone (14). Fresh leaf sample of about 30 mg was ground using 80% acetone (5 ml) with mortar and pestle and centrifuged at 10000 rpm for 10 min (4 °C). The supernatant was collected in a clean test tube while the pellet was re-suspended in 5 ml of 80% acetone. It was again centrifuged at 10000 rpm for 10 min and the supernatant was then used to measure the absorbance at 645 and 663 nm. Total chlorophyll and carotenoid was calculated using the given equations;

Total chlorophyll (mg/gm) = [(20.2(Ab645) + 8.02(Ab663)) × volume (ml)] ÷ [weight (gm) × 1000]

where Ab645 is the absorbance at 645 nm and Ab663 is the absorbance at 663 nm.

Carotenoid (mg/g) = [(7.6(Ab480) – (1.49(Ab510)) × volume (ml)] ÷ [weight (gm) × 1000]

where Ab480 is the absorbance at 480 nm and Ab510 is the absorbance at 510 nm.

Estimation of protein

Estimation of protein was done using Lowry's method (15) following the standard methodology (16) with modifications. The dried leaf powder of 50 mg was homogenized using phosphate buffer. After centrifugation at 10000 rpm for 10 min, 1 ml of the supernatant was added to another test tube to which 2 ml of Biuret reagent was added. It was then incubated for 10 min and 0.2 ml of FC reagent was added and the reaction mixture was kept in dark for 30 min after which absorbance was taken at 660 nm. BSA was used as standard. The amount of protein present in the sample was calculated using the standard graph obtained from BSA.

Quantification of Andrographolide

Extraction and quantification of andrographolide content was carried out following the standard methodology (17) with some modifications. Dried leaf powder of about 0.5 gm was extracted with methanol (25 ml) and was then incubated overnight at 20 °C on a rotary shaker (100 rpm). After filtration, the filtrate was air-dried and residue gained was re-dissolved in 2 ml of HPLC grade methanol. The HPLC system used was Waters 510 series equipped with Waters 486 series detector with column (250 mm × 4.6 mm) and detector wavelength was adjusted at 230 nm. The mobile phase comprised of acetonitrile: water (70:30 v/v) and the flow rate was 1.0 ml/min. Injection of aliquots of about 20 µl was carried out at 26 °C column temperature into the HPLC. Authentic, HPLC grade (purity 96%) andrographolide was obtained from Natural Remedies Pvt. Ltd., Bangalore, India.

Preparation of extract

1 gm of leaf powder after suspending in 10 ml of methanol was incubated for 3 hrs undisturbed. The set-up was then filtered followed by evaporation of the solvent by keeping in a boiling water bath. The extract was diluted to get 14 mg/ml concentration in the test samples that were further used for the estimation of total phenol and flavonoid content and also for antioxidant assays (18, 19).

Determination of Total Phenolic Content (TPC)

Folin-Ciocalteu method was used for TPC estimation (20). Plant extract (100 µl) was made up to 0.5 ml, to which 10% of 2.5 ml FC reagent was poured gently. This was followed by the addition of 2 ml Na_2CO_3 solution (7.5%) and incubation for 30 min in dark. Gallic acid was used in standard preparation and the wavelength was measured at 760 nm in the spectrophotometer.

Determination of Total flavonoid content (TFC)

Total flavonoid content was estimated (20) with some modifications. 4 ml of distilled water was added to 100 µl of plant extract that was made up to 1 ml initially. 0.3 ml of 5% $NaNO_2$ was added to the

solution followed by 5 min incubation at room temperature. 0.3 ml of 10% $AlCl_3$ was poured dropwise into the reaction mixture and kept undisturbed for 5 min at room temperature. This was followed by the addition of 2 ml of 1M NaOH and the final volume was made up to 10 ml. Quercetin was used in standard preparation and the absorbance was read at 510 nm.

Antioxidant Activity

Phosphomolybdate assay

The antioxidant activity of phosphomolybdate was performed (21) with slight modification. The reagent solution consists of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. To the plant extract (100 µl) made up to 0.3 ml, 3 ml of reagent solution was poured gently and was kept in the hot water bath for 90 min (95 °C). After cooling, the absorbance was measured at 695 nm.

Metal chelating assay

Metal chelating activity was performed (22) with slight modification. 1 mL $FeSO_4$ (0.1 mM) and 2 ml Ferrozine (0.25 mM) was added to the plant extract (50 µl) that was initially made up to 1 ml using methanol. The mixture was incubated for 10 min and the absorbance was measured at 562 nm. EDTA was used as standard.

$$\% \text{ metal chelating activity} = [(Ab(c) - Ab(s)) / (Ab(c)] \times 100$$

where Ab(c) is the absorbance of control without plant extract and Ab(s) is the absorbance of the sample with plant extract.

Radical scavenging activity using DPPH

Radical scavenging activity of sample extracts were determined by α-diphenyl –β-picrylhydrazyl (DPPH) method (23) where DPPH reagent (0.1 mM) of 1 ml was added to plant extract (50 µl) that was initially made up to 2 ml using methanol. The reaction mix was incubated for 15 min and then the absorbance was read at 517 nm. Ascorbic acid was used in the preparation of standard and 3 ml of methanol served as blank.

$$\% \text{ radical scavenging activity} = [(Ab(c) - Ab(s)) / (Ab(c)] \times 100$$

where Ab(c) is the absorbance of control without plant extract and Ab(s) is the absorbance of the sample with plant extract.

Statistical analysis

All the experiments were done in triplicates and the results were expressed as mean ± standard error. Duncan multiple range test (DMRT) is used where means not sharing a common single letter found to be significantly different at $p \leq 0.05$.

Results and Discussion

Total chlorophyll, carotenoid and protein content

Chlorophyll and carotenoid are organic pigments present naturally in plants that give the compounds their specific colour and play an important role in photosynthesis (24). Table 1 presents the concentration of total chlorophyll, carotenoid and protein after heavy metal treatment in *A. paniculata*. The control (10.45 mg/gm of the fresh leaf) has shown the maximum chlorophyll content. A decline in the total chlorophyll concentration was seen in all heavy metal treated samples and the lowest concentration was observed in 100 mM Sn treatment (2.03 mg/gm of the fresh leaf). In a study conducted (25), total chlorophyll concentration was found to decrease from the control after copper treatment in *Gynura procumbens*. Reduction in the chlorophyll content can be because of the inhibiting action of heavy metals on chlorophyll biosynthesis enzymes and thereby obstructing plant metabolic processes leading to chlorophyll degradation (25, 26).

Carotenoids are the pigment that give red colour to the tomatoes and has a symmetrical tetraterpene structure (24). In the present study, a decrease in the carotenoid concentration was seen in all heavy metal treated plants except 50 mM Sn treated plants (Table 1). The least carotenoid content was seen in 50 mM cobalt (1.32 mg/gm of the fresh leaf) followed by 100 mM tin (1.64 mg/gm of the fresh leaf) treatments. In a study (27), the concentration of chlorophyll and carotenoid was increased in the shoots of 250 µmol L^{-1} Cu treated *Solanum cheesmaniae* when compared with the control. Similar to our results, one study (28) demonstrated that mixed HM (Cd, Cr, Cu, and Zn) treated hybrid plants (DN 034, TN 074, TD 225) has shown a decline in the carotenoid content. Heavy metal accumulation in leaves reduces photosynthesis indirectly by the reduction of photosynthetic pigments. These pigments can be measured easily and are used frequently in the determination of stress (29).

Table 1. Impact of heavy metal stress (Cu, Sn and Co) on chlorophyll, carotenoid and protein content.

Heavy Metal Concentration (mM)	Total chlorophyll (mg/gm fresh leaf)	Carotenoid (mg/gm fresh leaf)	Protein (mg/gm DW)
Control	10.45 ± 0.30a	2.93 ± 0.09a	13.51 ± 0.15bc
Cu 50	7.70 ± 0.04ab	2.02 ± 0.01b	21.07 ± 0.13a
Cu 100	7.94 ± 0.01ab	2.06 ± 0.01b	22.03 ± 1.10a
Sn 50	9.14 ± 0.18a	3.36 ± 0.05a	15.53 ± 1.03b
Sn 100	2.03 ± 0.05c	1.64 ± 0.00b	12.20 ± 1.16c
Co 50	5.11 ± 2.55b	1.32 ± 0.66b	11.26 ± 0.11c
Co 100	7.62 ± 0.03ab	2.10 ± 0.01b	8.62 ± 0.03d

All samples were analyzed in triplicates and the results are represented as mean ± standard error. Means not sharing a common single letter found to be significantly different at $p \leq 0.05$ where Duncan Multiple Range Test (DMRT) was used.

Proteins are macromolecules that consist of amino acid units as their building blocks and are one of the most abundant molecules present in living organisms (30). In the present study, copper increased the protein content while cobalt decreased the protein content in *A. paniculata* in comparison to the control. Variations in the protein content from the control (13.51 mg/gm) were seen in Tin treatments, where 50 mM Sn treated plants (15.53 mg/gm) have shown an increase while 100 mM Sn treated plants (12.2 mg/gm) have shown a reduction in the protein content (Table 1). Total chlorophyll and protein content was decreased after the application of cobalt stress at a concentration of 50 to 250 mM in *Raphanus sativus* (31). In *Lycopersicon esculentum*, a reduction in the level of protein and non-protein

nitrogen was observed after providing cobalt stress (32). Proteins are the primary target of heavy metals such that physiological functions get impaired by them either by forming a complex with proteins or by displacing essential ions from metalloproteins (33).

Andrographolide content

Andrographolide is a principal medicinal component and an important secondary metabolite produced in *A. paniculata* that possess many pharmacological activities (12). In the present study, heavy metals resulted positively in the andrographolide content in *A. paniculata* except 100 mM tin treated plants (5.79 mg/gm of DW) with a slight decrease in the concentration from that of control (6 mg/gm of DW). The highest level of andrographolide was seen in 50 mM copper (8.5 mg/gm of DW) and 50 mM tin (8.1 mg/gm of DW) treatments respectively (Fig. 1). In a study conducted (34), 4 fold rise in the andrographolide content was seen after treatment with an abiotic elicitor, copper sulphate at 100 µM to 500 µM concentrations. $CuCl_2$ treatments have resulted in the elicitation of andrographolide in the suspension culture of *A. paniculata* (35). Significant production of andrographolide of about 3-7 fold increase was observed after treatment with elicitors like silver nitrate, L- aspartic acid and methyl jasmonate (36). Elicitor induced stress might either result in the activation or inactivation of certain genes that can bring about changes in the biosynthetic pathway of many secondary metabolites (37).

Total Phenolic Content (TPC) and Total flavonoids content (TFC)

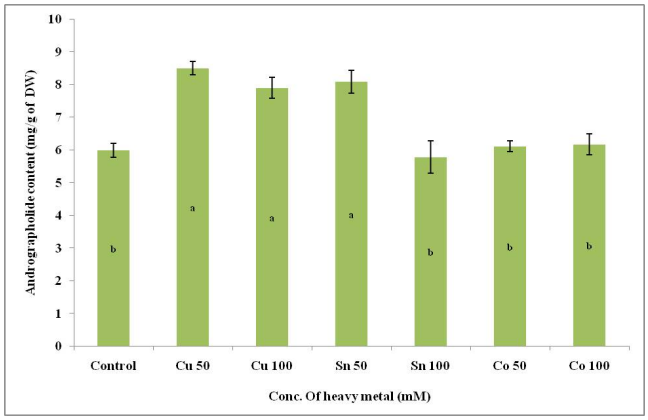

Fig. 1. Effect of heavy metal stress (Cu, Sn and Co) on the andrographolide content in *A. paniculata.* All samples were analysed in triplicates and the results are represented as mean ± standard error. Means not sharing a common single letter found to be significantly different at $p \leq 0.05$ where Duncan multiple range test (DMRT) was used.

Phenolic compounds or polyphenols are found abundantly in the plant kingdom and are present in almost all plant organs. These compounds are produced due to secondary metabolism in plants where some of them are associated with antioxidant and free radical scavenging activities (38). Table 2 presents the amount of TPC and TFC in *A. paniculata* after heavy metal treatment. Tin and cobalt had a positive impact on TPC in *A. paniculata* when compared to the control. 50 mM cobalt treated plants (17.21 mg/gm of extract) have shown the maximum content of TPC and the least concentration was seen in 100 mM copper treatment (10.96 mg/gm of extract). Copper treatment at 10, 20 and 50 ppm in *Zea mays* had increased total phenolic content in comparison to the control. Phenolic compounds are considered to be one among the stress responses produced by plants for their adaptation. These compounds can trap alkoxyl radicals and inhibit lipid peroxidation (39).

Flavonoids are low molecular weight phenolic compounds. They represent the commonly distributed plant phenolic group with two aromatic ring structures linked through three carbons (38). The $AlCl_3$ method was used for the estimation of TFC in the present study. Tin treated plants have shown an increase while copper treated plants have shown a decrease in the TFC in *A. paniculata*. The highest and lowest concentration of TFC was observed in 50 mM cobalt (6.97 mg/gm of extract) and 100 mM copper (3.05mg/gm of extract) treatments respectively.

An increase in the amount of TPC and TFC was observed in copper and cadmium treated *G. Procumbens* in comparison to the control (25). In a study (27), flavonoid content in the shoots was found to increase in 250 µmol L^{-1} Cu treated *Solanum lycopersicum* while it was found to decrease in 250 µmol L^{-1} Cu treated *Solanum cheesmaniae*. An increase in TFC and TPC was observed in *Salvia officinalis* under 500 µM $CoCl_2$ treatments as compared to control (40). In plants, flavonoids are located in mesophyll cells that act as a defense system against different stresses. Antioxidant effects are mediated by the functional hydroxyl groups present in them which otherwise would result in radical generation leading to the damage of targeted biomolecule (41).

Table 2. Impact of heavy metal stress(Cu, Sn and Co) on Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) in *A. paniculata*

Heavy Metal Concentration (mM)	TPC (mg/gm of extract)	TFC (mg/gm of extract)
Control	11.35 ± 0.65c	4.06 ± 0.13c
Cu 50	12.45 ± 0.46bc	3.73 ± 0.20c
Cu 100	10.96 ± 0.44c	3.05 ± 0.06d
Sn 50	14.03 ± 1.03b	4.93 ± 0.20b
Sn 100	12.58 ± 0.26bc	5.11 ± 0.04b
Co 50	17.21 ± 0.93a	6.97 ± 0.19a
Co 100	11.90 ± 0.83bc	3.90 ± 0.25c

All samples were analyzed in triplicates and the results are represented as mean ± standard error. Means not sharing a common single letter found to be significantly different at $p \leq 0.05$ where Duncan Multiple Range Test (DMRT) was used.

Phosphomolybdate, metal chelating and DPPH radical scavenging activity

Antioxidants can inhibit cell damage caused by free radicals mostly by scavenging and neutralizing them. A free radical is a molecular species that can exist independently with an unpaired electron which makes them unstable and highly reactive (42). Phosphomolybdate assay was used in the evaluation of antioxidant activity in *A. paniculata* after providing heavy metal stress as shown in Table 3. Here phosphomolybdate ion gets reduced and results

in a green coloured complex in the presence of an antioxidant (23). The data were represented as mg/gm of extract equivalent to ascorbic acid. Maximum antioxidant activity was seen in 100 mM cobalt (43.72 mg/gm equivalent to ascorbic acid) treated plants. Tin treated plants have shown an increase in the activity and the least activity was found in 50 mM cobalt (64.48 mg/gm equivalent to ascorbic acid) treated plants. Cu at 200 ppm had no impact on the antioxidant ability of *Ocimum basilicum* while comparing with the control (43).

Fe^{2+}/Ferrozine method was used in the determination of metal chelating activity (Fig. 2). Fe^{2+} gets quantitatively chelated by Ferrozine which results in the formation of Fe^{2+}-Ferrozine complex (44). The highest activity was observed in 50 mM copper (57.15%) treated plants followed by control (56.55%) while the lowest activity was seen in 50 mM cobalt (51.61%) treated plants. No significant variations in the antioxidant activity were seen in samples in this method when compared with untreated control plants. *Clitocybe geotropa* was detected with 65.6 mg/kg of copper, 0.5 mg/kg of cobalt and other heavy metals, whose chelating effect was 28, 37 and 43% at 1, 2 and 4 mg/ml of sample concentration respectively (45). Here the chelating ability of the extracts was found to increase with the elevating concentration.

Table 3. Impact of heavy metal stress (Cu, Sn and Co) on the phosphomolybdate activity in *A. paniculata*

Concentration (mM)	Phosphomolybdate assay (mg/g equivalent to ascorbic acid)
Control	60.29 ± 1.96ab
Cu 50	63.84 ± 2.29a
Cu 100	55.71 ± 0.58bc
Sn 50	53.99 ± 0.54c
Sn 100	53.94 ± 1.97c
Co 50	64.48 ± 2.23a
Co 100	43.72 ± 0.12d

All samples were analyzed in triplicates and the results are represented as mean ± standard error. Means not sharing a common single letter found to be significantly different at $p \leq 0.05$ where Duncan Multiple Range Test (DMRT) was used.

DPPH radical scavenging assay was performed for the estimation of antioxidant activity in *A. paniculata* after heavy metal treatment as presented in Fig. 3. This assay is assessed based on the decline in DPPH resulting in a colour change to yellow from purple (23). Copper treated plants have shown a decline in antioxidant activity while tin treated plants have shown an increase. In comparison with the control (55.73%), the highest and the lowest DPPH radical scavenging activity was observed in 50 mM Sn (63.8%) and 50 mM Cu (34.8%) treated plants respectively. In a study (40), a 57.8% increase in the antioxidant activity was observed after 500 µM $CoCl_2$ treatment in *Salvia officinalis* and in a study (46), after 15 days exposure of *Colobanthus quitensis* to 150 and 300 µM of copper sulphate, an induction in antioxidant activity by DPPH radical scavenging was observed in *in vitro* culture. A rise in DPPH activity was observed after 70 and 140 mg/L copper treatment in *G. procumbens* (25). The defense mechanism gets activated in plants when they encounter metal stress mainly in terms of antioxidant enzymes (47). Production of ROS is evident in all the organisms as a consequence of HM stress which is comparatively more reactive than the molecular oxygen. This toxicity from HM also results in the induction of cellular injury in cellular components like proteins, DNA etc. Total antioxidant capacity can be evaluated by various types of methods in plants. Nowadays, many DNA based techniques are useful in assessing variations in DNA as a result of genotoxic agents related exposure (48).

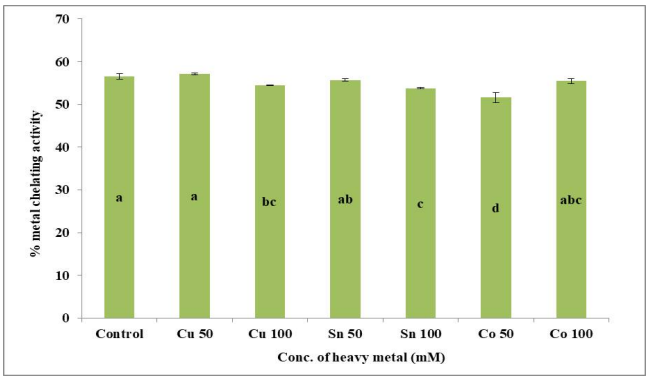

Fig. 2. Effect of heavy metal stress (Cu, Sn and Co) on metal chelating activity in *A. paniculata.* All samples were analysed in triplicates and the results are represented as mean ± standard error. Means not sharing a common single letter found to be significantly different at $p \leq 0.05$ where Duncan multiple range test (DMRT) was used.

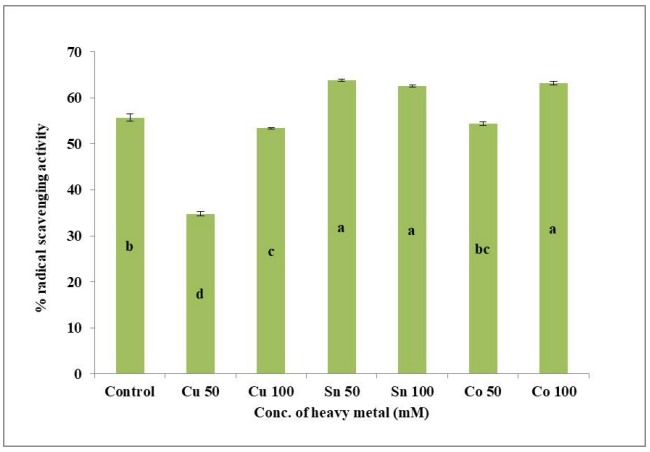

Fig. 3. Effect of heavy metal stress (Cu, Sn and Co) on radical scavenging activity in *A. paniculata.* All samples were analysed in triplicates and the results are represented as mean ± standard error. Means not sharing a common single letter found to be significantly different at $p \leq 0.05$ where Duncan multiple range test (DMRT) was used.

Conclusion

From the results it is clear that HM treatment has induced stress that resulted in the variations in andrographolide content, biochemical parameters, and anti-oxidant activity in *A. paniculata*. Being one among the major secondary metabolite produced by this herb, andrographolide possesses many biological activities. In the present study, Copper stress has resulted in the maximum production of andrographolide in *A. paniculata* after HM treatment. Herbal products that come from the raw or processed part of the plants are being widely used by the

population. A study conducted in Asian patent medicines, twenty-five percentages of the products were found to contain high HM content (49). Since many plants and their products are an important source of medicine, the removal of HM during herbal formulation has to be taken care properly. The presence of HM in the medicinal plant products can reduce their activity. Metabolomics coupled with molecular biology techniques can provide more insights into secondary metabolite production after heavy metal stress.

Acknowledgements

The authors are grateful to HOD, Department of Life Sciences, CHRIST (Deemed to be University) for providing an opportunity to work and successfully complete the project.

Authors' contributions

The current study was designed by PN, AA collected the samples, performed the experiments, analysed the data and wrote the manuscript. PN edited the manuscript, communicated with the journal and resolved the reviewer's comments.

Conflict of interests

The authors declare they have no conflict of interests.

Supplementary files

Fig. 1. *Andrographis paniculata* acclimatized in poly-house.

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