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Effect of heavy metal elicitation on antioxidants and andrographolide content in cell suspension cultures of *Andrographis paniculata*

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Abstract

Andrographolide, a bicyclic diterpene from *Andrographis paniculata* is of immense pharmaceutical importance. *A. paniculata*, an annual herb from Acanthaceae is widespread in the Indian subcontinent. Heavy metals act as abiotic elicitors. The study deals with the effect of mercury (Hg), cadmium (Cd) and arsenic (As) on andrographolide content, phenols and flavonoids and each of their correlation with the metal chelating and radical scavenging activity, in cell suspension cultures of *A. paniculata*. Andrographolide was estimated using HPLC, while other estimation methods were used for other metabolites. Four different concentrations of each of the heavy metal salts $CdCl_2$, As_2O_3 and $HgCl_2$, were administered in liquid MS media containing 1 g of cells. Media without any metal served as control. Higher concentrations of Cd and As imparted a positive effect on andrographolide content, Hg imparted a negative effect. The cells were most sensitive to Hg and most tolerant to Cd. Cd could be the best choice as an elicitor for increased production of andrographolide. While phenols show a positive correlation with antioxidants, flavonoids and andrographolides do not show a positive correlation with antioxidants.

Keywords

Andrographis paniculata; andrographolide; arsenic; cadmium; flavonoids; mercury; phenols

Introduction

Andrographis paniculata has numerous pharmacologically important phytochemicals such as labdane diterpenes, quinic acid derivatives, flavonoids, xanthones and nor-iridoids (1-4). The herb is well known for its reported antibacterial, antifungal, antimalarial, antileishmanial, filaricidal, antioxidant, anti-inflammatory, antidiabetic, anti-diarrhoeal, antifertility, antivenom, vasorelaxant, hepatoprotective, immunomodulatory and anti-cancerous effects (5, 6). The main secondary metabolite of the plant is andrographolide, a bicyclic diterpene, which is reported to have anti-inflammatory (7), apoptotic (8), anti-proliferative (9) and anti-viral effects (10). The metabolite also has been proven to be effective against leukaemia (11), carcinoma (12) and hepatoma (13). Alcoholic extracts of leaves, stem and roots of *A. paniculata* reportedly contain over 10 flavonoids and 20 diterpenoids (14). Andrographolide makes up about 4 % of dry weight in the whole plant, 1.2 % in stem and 0.5-6 % in leaves (16, 17). Deoxyandrographolide, 14-deoxy-11,12 didehydroandrographolide, neoandrographolide and isoandrographolide are the other diterpenoids specific to *A. paniculata* (16, 18). The plant also has a stockpile of flavonoids (19).

Elicitors are often used in *in-vitro* cell suspension cultures to enhance secondary metabolite production (20). They often induce stress and activate biochemical pathways (21). Naturally, in response to abiotic stresses induced by herbivory-induced wounds, salinity, heavy metals, drought, temperature, lack or excess of micronutrients etc., Jasmonic acid (JA) serves as a signalling molecule to induce downstream reactions related to plant defense (22). JA was particularly effective in enhancing andrographolide content (23). Abiotic elicitors often include inorganic compounds and heavy metal ions (24). *In-vitro* work does exist on the use of abiotic elicitors to produce pharmacologically important terpenoids in *A. paniculata* (25, 26). Cd reportedly resulted in a four-fold increase in the andrographolide content relative to control (25). Arsenic triggered the production of relatively higher amount of andrographolide (27). Ag treatment in *A. paniculata* saplings also has shown to amplify andrographolide production (28).

While most of the previous studies have focused on the effect of heavy metal elicitors on andrographolide content alone, the current study aims to understand the effect of Cd, As and Hg on all five biochemical components: andrographolide, phenols, flavonoids, radical scavenging activity and metal chelating potential in cell suspension cultures of *A. paniculata*; the hypothesis that is being tested in the study is if andrographolide production is influenced by the amount of phenols, flavonoids and other antioxidants that the cells produce. The study adds new knowledge and offers insights into how increase in heavy metal concentrations in the liquid medium can influence the dynamics of phenols and flavonoids, the potential non-enzymatic antioxidants and their collective effect on andrographolide; measuring the heavy metal concentration in callus was not the objective of the study. This work particularly also takes into consideration the aspect of productivity for all the metabolites studied.

Materials and Methods

Establishment of callus cultures from A. paniculata

A. paniculata leaves were collected from the plants grown in the green house of CHRIST (Deemed to be University), Bangalore and used as explants. They were surface sterilized with 2 % NaOCl for 7 min, 0.1 % $HgCl_2$ for 30 s and washed with sterile distilled water five to six times. Young leaves of 0.5 cm^2 were cultured on MS media supplemented with various concentrations of plant growth regulators (PGR)s. Media supplemented with Picloram (1 mg/L) and Thidiazuron (1 mg/L) was chosen for callus induction (27). The callus cultures were maintained at 25 ± 2 °C in 16-h photoperiod provided by 40 W white, fluorescent tubes (2000 lux).

In-vitro cell suspension culture

Initiation of cell suspension cultures was done by suspending 1 g of friable callus in 50 mL MS media supplemented with Picloram (1 mg/L) and Thidiazuron (1 mg/L), taken in 250 mL conical flasks. The cultures were incubated on a shaker maintained at 100 rpm at 25±2 °C under a 16-h photoperiod provided by 40 W white, fluorescent tubes (2000 lux).

Elicitation by heavy metals

Heavy metal salts $CdCl_2$, As_2O_3 and $HgCl_2$ were evaluated for their influence on andrographolide content, total phenolics, total flavonoids, radical scavenging potential and metal chelating potential in *A. paniculata* cell suspension cultures after 28 days. 1 g of friable callus was taken in 50 mL liquid MS media supplemented with Picloram (1 mg/L) + Thidiazuron (1 mg/L). 1000 µM/mL stock of each heavy metal salt was prepared. Previous studies have administered lower concentrations of heavy metals, particularly on the 20^{th} to 24^{th} day of culturing. However, in the present study, four different concentrations (25 µM, 50 µM, 75 µM, 100 µM) of each heavy metal were added each, aseptically, in triplicates, to the liquid medium using micropipettes on the very first day; the rationale was to study the impact of these heavy metals from day 1, considering natural conditions of heavy-metal-contaminated-soils. The flasks with the cell suspensions were then incubated on a shaker incubator for 28 days in the same controlled conditions used for initiating and maintaining cell suspension cultures. After 28 days, the cells were harvested for further studies.

Extraction of andrographolide and quantification using HPLC

The extraction and quantification of andrographolide was performed following the method of Zaheer and Giri (30). 0.1 g of dry weight of powdered cells was taken in a falcon tube and a proportional amount of methanol was added to give a 1 % solution. It was incubated at room temperature for 1 h and sonicated for 30 s. Whatman filter paper no. 41 was used to filter the homogenate. Before being injected into HPLC, the homogenate was filtered again using a 0.45 µm membrane filter. Shimadzu chromatographic system equipped with the Phenomenex column 18 (5 µM, 4.6 X 250 mm) was used for HPLC. Acetonitrile: Water (40:60) served as the mobile phase and 1 mL/min flow rate was maintained. Elution was monitored at 230 nm. 10 µL of the samples were injected. Standard andrographolide (1 mg/mL) was prepared using HPLC-grade methanol and was used for analysis. Triplicates were used for each treatment. Andrographolide content was estimated by calculating the area under the peak using the software Empower Pro and expressed in µg/g dry weight.

Total phenolic content

Folin-Ciocalteau (FC) reagent assay with gallic acid as a standard was followed to estimate the total phenol content (31). 0.1 g of dry powdered cells was suspended in 1 mL methanol (80 %) taken in a micro vial and centrifuged for 5 min at 10000 rpm. In a different vial the supernatant was collected and let to evaporate. Once dried, the residue was suspended in 1 mL distilled water. 0.1 mL of the homogenate was taken in another micro vial and mixed with 0.6 ml distilled water. Thereafter, 0.1 mL FC reagent was added. Three min later 0.4 mL of 20 % Na_2CO_3 was added and mixed well. The micro vials were then kept in a hot water bath for a minute and allowed to cool. Absorbance of the homogenate was then measured at 765 nm using the microplate reader (BIO-RAD, iMARK™, Japan). Experiments were done in triplicates.

Total flavonoid content

Aluminium chloride assay described in Zhishen et al (32) was followed with quercetin as the standard. A 0.1 g of dried, powdered cells was suspended in 1 mL methanol and filtered. The volume of the filtrate was made up to 1 mL using the same solvent. A 0.1 mL aliquot of the filtrate was transferred into a separate micro vial and combined with 0.3 mL of 80 % methanol, 0.02 mL of 10 % aluminum chloride, 0.02 mL of 1 M potassium acetate, and 0.56 mL of distilled water. Distilled water served as a blank. Absorbance was measured at 415 nm using the microplate reader (BIO-RAD, iMARK™, Japan). Experiments were done in triplicates.

Antioxidant and free radical scavenging activity

DPPH assay described by Shen et al was used (33); high radical scavenging potential is indicated by lower absorbance values. A 0.1 g of dried powdered cells was suspended in 1 mL of methanol and filtered; the filtrate volume was made up to 1 mL using methanol. In another micro vial, 20 µL of the filtrate was taken and made up to 0.6 mL with methanol. Then, a volume of 0.2 mL of DPPH was then added and incubated in dark for 20 min. A volume of 20 µL of methanol instead of the extract served as the control. After incubation, the absorbance was measured at 517 nm using the microplate reader (BIO-RAD, iMARK™, Japan). DPPH activity was measured as per the formula mentioned in Shen et al (33).

Metal chelating potential

Ferrozine assay described in (34) was followed. A 0.1 g of dry powdered cells was suspended in 1 mL of methanol and kept at room temperature for an hour. After an hour 0.04 mL of the filtered extract was taken in a micro vial and 100 µM $FeSO_4$ plus 0.25 mM ferrozine were added. The vials were incubated for 10 min. Absorbance was measured at 562 nm using the microplate reader (BIO-RAD, iMARK™, Japan). Metal chelating activity was estimated using the formula mentioned in Chew et al. (34).

Statistical analysis

All the experiments were performed in triplicates. The results obtained are represented as mean ± SE of triplicate of experiments. The data of the experiments were statistically analyzed using one-way ANOVA. Duncan's multiple range test (DMRT) using IBM's SPSS software version 24 was used for significance.

Results and Discussion

Callus initiation and proliferation

Leaf explants cultured on semi-solid MS medium supplemented with 1 mg/L Picloram and 1 mg/L Thidiazuron (TDZ) successfully produced a substantial amount of friable callus. The hormonal combination of Picloram and TDZ proved effective in promoting callus formation, highlighting their synergistic role in cell dedifferentiation and proliferation. The callus was subsequently transferred to liquid MS medium with the same hormonal combination to establish cell suspension cultures, which were further propagated through regular subculturing. This approach ensured the generation of a stable and uniform culture system suitable for downstream elicitation studies. The consistent results demonstrate the reliability of this protocol for maintaining and utilizing *A. paniculata* cell cultures in various experimental setups.

Effect of Cd, As and Hg on biomass and andrographolide content

The presence of heavy metals, regardless of the concentration, had a significant negative impact on biomass accumulation. As the concentration of heavy metals increased, the extent of biomass accumulation progressively decreased. Cd, As and Hg at a concentration of 100 µM resulted in the reduction of dry weight by 45.07 %, 49.8 % and 44.15 % respectively, compared to the control (Fig. 1). Control had the highest biomass and andrographolide content with highest productivity. Productivity is of the order: Control > As 25 µM > Cd 50 µM > Cd 75 µM > As 50 µM > Cd 100 µM. Cd, even at the highest concentration of 100 µM, did not show any negative effect on andrographolide content (0.18-fold increase). Notably, higher flavonoid content and metal chelating activity, both of which serve as antioxidants, were seen at higher concentrations of Cd (Table 1 and Fig. 2). The results are consistent with Gandi et al. (25) who reported a four-fold increment in andrographolide upon treatment with $CdCl_2$. Moreover, the accumulation and translocation of any heavy metal is largely dependent on the genotype of *A. paniculata.* Andrographolide content increased with the increase in As concentration, peaking at 75 µM (0.17-fold increase compared to control) (Fig. 1) and dipping drastically at 100 µM. The negative effect of As^{3+} on andrographolide content could be attributed to the fact that As^{3+} shows high affinity for dithiol groups, especially enzymes where cysteine residues are close to each other (35); proteomics studies may be needed to corroborate the

Table 1. Effect of Cd, As and Hg on radical scavenging and metal chelating potential of cell suspension cultures of *A. paniculata*

Metal	Concentration µM	Radical scavenging activity	Metal Chelating activity
Control	0	79.83±0.56d	70.05±0.53d
Cd	25	91±0.57a	75.8±0.77c
	50	82.5±0.4b	80.8±0.6b
	75	52.7±0.5g	84.1±0.4a
	100	39.9±1.2j	75.5±0.5c
As	25	69.8±0.8e	75.2±1.2c
	50	49±0.88h	77±0.7c
	75	40.5±0.5j	59.3±0.8f
	100	30.1±1.2k	56.5±0.4g
Hg	25	77.6±0.4d	75.6±0.6c
	50	66.5±0.9f	81.1±0.4b
	75	47.4±0.8h	65.6±0.3e
	100	44.7±0.6i	52.6±0.4h

Data in the figure represent the mean value of the triplicates. Values after ± indicate the standard error. Mean values with common letters are not significantly different at P ≤ 0.05 according to Duncan's multiple range test (DMRT)

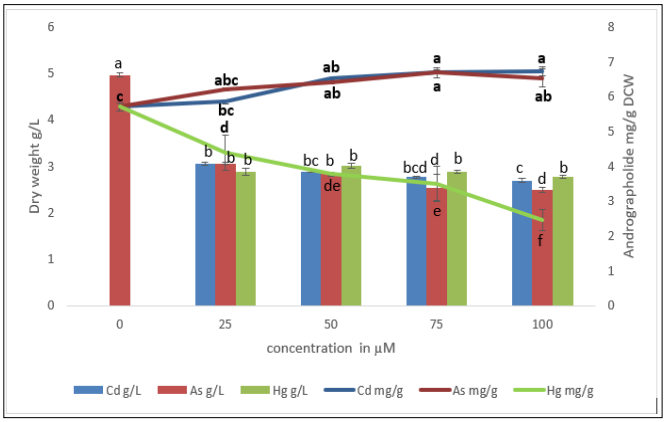


Fig.1. Effect of Cd, As and Hg on dry weight and andrographolide content in cell-suspension cultures of *A. paniculata.*

Data in the figure represent the mean value of the triplicates. Values after ± indicate the standard error. Mean values with common letters are not significantly different at P ≤ 0.05 according to Duncan's multiple range test (DMRT)

assumption. Nevertheless, the results align with those of previous research which demonstrated that *A. paniculata* plants treated with 7 µM of As_2O_3 resulted in a relatively high andrographolide compared to control (27); the current study reported a much higher andrographolide (6.68 ± 0.14 mg/g DW). The trend could also be attributed to the effect of As^{3+} on metal chelating activity, which dips post 50 µM of As (Table 1), indicating the diminishing ability of cells to synthesize enough antioxidants to chelate metals- more work is needed to consolidate these claims in the study. Hg affected the andrographolide content negatively; 100 µM of Hg showed a 42 % decrease in andrographolide content compared to the control. With the increase in Hg concentration, there was a proportional reduction in andrographolide content. The results could be attributed to the ability of Hg to bind with the sulfhydryl group of cysteines which forms a part of key plant metabolic enzymes (36) and block aquaporins (37).

Effect of Cd, As and Hg on total phenolic content and total flavonoid content

Phenol content peaked at 50 µM of Cd (91 %-increase compared to control) but dipped at higher concentrations (Fig. 3), whereas there was a progressive increase in the total flavonoid content - a 33 % increase compared to control at 100 µM (Fig. 2). These findings are in accordance with the results obtained by Manquián -Cerda et al (38). Arsenic imparted a negative effect on phenols with an increase in its concentration. At the highest concentration of 100 µM, a 0.74-fold decrease in TPC (Fig. 3) compared to control was seen. TFC increased initially, peaking at 50 µM of As - a 1.97-fold increase compared to control, only to dip at higher concentrations (Fig. 2). Notably, the effect of As on TPC differs from that on TFC; phenols show a linear decrease with increase in As concentration, while flavonoids show an initial increase followed by a dip. This could be because of increase in the expression of chalcone synthase, the key enzyme involved in the biosynthesis of flavonoids from 4-coumaryl coenzyme A, upon exposure to As^{3+} (39). Hg showed a similar effect on phenols and flavonoids respectively as the concentration of the metal increased there was a gradual reduction in both the components- 11.38-fold decrease in TPC and 2.63-fold decrease in TFC compared to the control at 100 µM Hg (Fig. 2). Siatka et al. (40) had reported a reduction in phenol content when Hg concentration higher than 20 µM was administered in cell suspension cultures of *Angelica archangelica*. Net reduction in phenol content upon treatments with higher concentration of Hg has also been reported in *Mucuna pruriens* (41).

Effect of Cd, As and Hg on antioxidant activity

The cells exhibited distinct trends in response to cadmium (Cd), arsenic (As) and mercury (Hg) with respect to radical scavenging potential and metal chelating activity. For radical scavenging potential, Cd at lower concentrations (25 µM and 50 µM) enhanced activity by 13.8 % and 3.1 %, respectively, compared to the control. However, at higher concentrations, this potential decreased, with the highest activity observed at 25 µM of Cd. This is consistent with studies on *in vitro* plantlets of blueberry, where radical scavenging potential increased at 50 µM of Cd (Table 1) (38). As and Hg showed similar trends in metal chelating activity, peaking at 50 µM with increase of 9.7 % and 15.6 %, respectively, compared to the control, but declining at higher concentrations. In contrast, Cd increased metal chelating activity consistently up to 75 µM (a 19.92 % increase compared to the control) before showing a decrease at higher concentrations. The patterns observed for andrographolide, a bicyclic diterpene synthesized through the mevalonate and non-mevalonate pathways, differed from those for phenols and flavonoids, which are products of the shikimic acid pathway. Since these pathways are unrelated, the trends for phenols and flavonoids were distinct from those seen for andrographolide (Table 1).

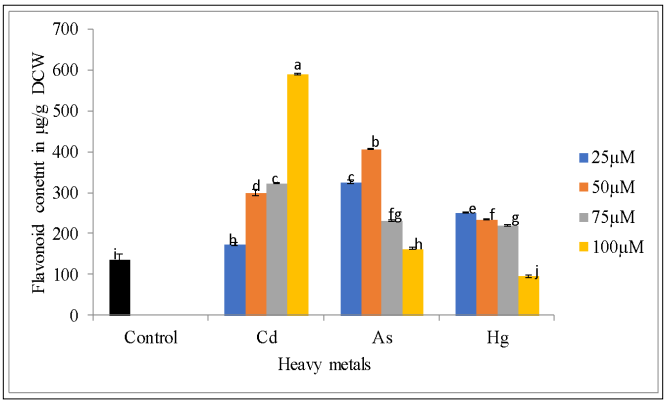


Fig. 2. Effect of Hg, As and Cd on total flavonoid content in cell suspension cultures of *A. paniculata.*

Data in the figure represent the mean value of the triplicates. Values after ± indicate the standard error. Mean values with common letters are not significantly different at P ≤ 0.05 according to Duncan's multiple range test (DMRT)

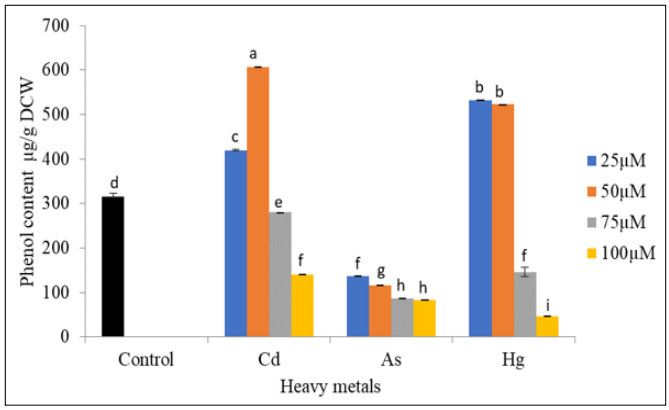
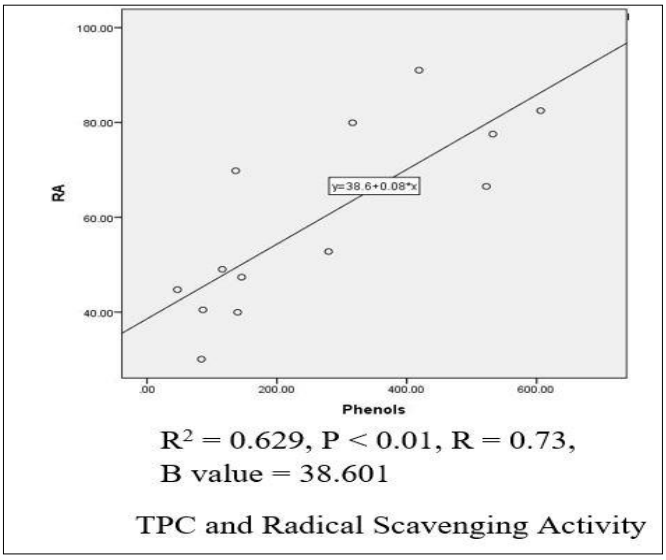


Fig. 3. Effect of Cd, As and Hg on total phenolic content in cell suspension cultures of *A. paniculata.* Data in the figure represent the mean value of the triplicates. Values after ± indicate the standard error. Mean values with common letters are not significantly different at P ≤ 0.05 according to Duncan's multiple range test (DMRT)

Correlation analyses

Correlation and linear response between total phenols, total flavonoids, andrographolide and antioxidant activities were calculated.

1. Linear and positive response between TPC and radical scavenging activity (R^2 = 0.63, R = 0.73, P < 0.73, B = 38.6), and TPC and metal chelating activity (R^2= 0.431, R = 0.656, P < 0.05, B = 62.67). A relatively high correlation of 73 % was seen between TPC and Radical scavenging activity (Fig. 4). Notably, relatively lower but positive correlation of 65.6 was seen with metal chelating activity. The results do not show as much correlation seen with previous reports (44-46). Nonetheless, there does exist a positive correlation (Fig. 4)
2. Linear and positive responses between TFC and antioxidants (Ferrozine assay) (R^2= 0.28, R = 0.529, P < 0.01, B = 60.64) confirms that flavonoids are acting as metal chelators too, although not in substantial proportion, with a correlation of 53 % (Fig. 5).
3. Linear and positive responses between andrographolide and antioxidants (Ferrozine assay) (R^2= 0.113, P < 0.01, R= 0.337, B = 55.7) confirms that the bicyclic diterpene shows a positive correlation of 33.7 % with antioxidant activity. The result is in line with previous observations (47-49) which have demonstrated the ability of andrographolide to act as a potent antioxidant (Fig. 6).
4. Linear and almost negative responses between TPC and TFC (R^2= 0.005, R = 0.071, P < 0.05, B= 278.437) confirms a moderate correlation of 7.1 with each other (Fig 7). The results of the current study are contrary to previous reports (44-47). It could be because not all phenols get converted to flavonoids. Chalcone synthase, the enzyme responsible for the biosynthesis of flavonoids is crucial for the conversion of phenols to flavonoids (50). Since this study involved treatment of heavy metals, it is possible to assume their deleterious effect on any of the enzymes involved in flavonoid biosynthesis; RT-PCR studies could shed some light in this regard.



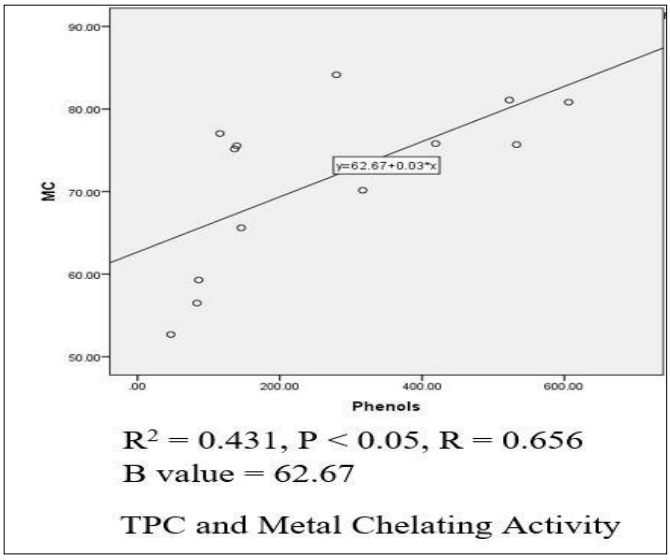
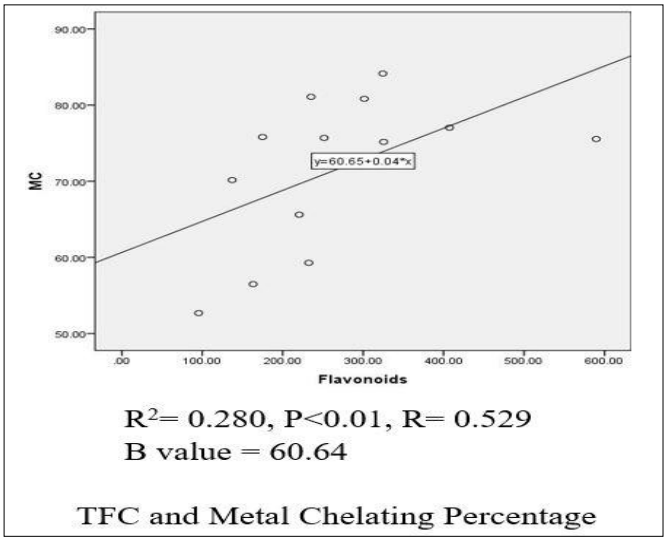


Fig. 4. Linear Regression Analysis of Total Phenolic Content (TPC) with antioxidant activities.

Linear regression showing TPC positively correlates with Radical Scavenging Activity (y = 38.6 + 0.08x, R^2 = 0.629, P < 0.01, B = 38.6) and Metal Chelating Activity (y = 62.67 + 0.03x, R^2 = 0.431, P < 0.05, B = 62.67). The B value represents the intercept, indicating baseline activity levels independent of TPC. The y-axis represents Radical Scavenging Activity (%) in the left graph and Metal Chelating Activity (%) in the right graph.



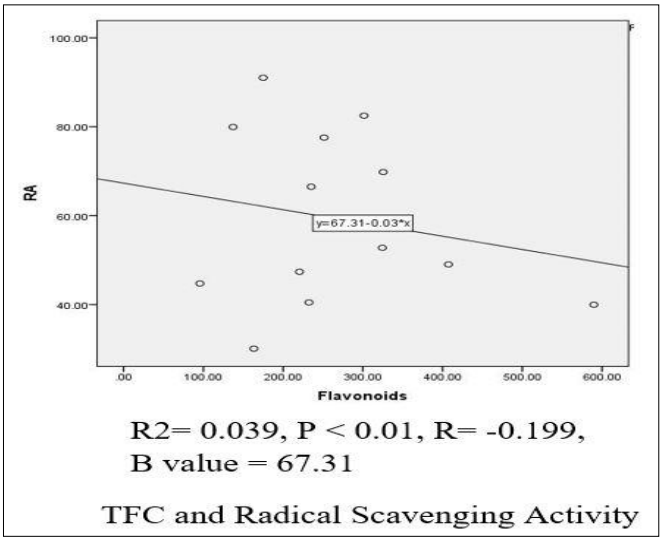


Fig. 5. Linear Regression Analysis of Total Flavonoid Content (TFC) with antioxidant activities.

Linear regression showing TFC positively correlates with Metal Chelating Activity (y = 60.65 + 0.04x, R^2 = 0.280, P < 0.01, B = 60.64) but negatively correlates with Radical Scavenging Activity (y = 67.31 - 0.03x, R^2 = 0.039, P < 0.01, B = 67.31). The B value represents the intercept, indicating baseline activity levels independent of TFC. The y-axis represents Metal Chelating Activity (%) in the left graph and Radical Scavenging Activity (%) in the right graph.

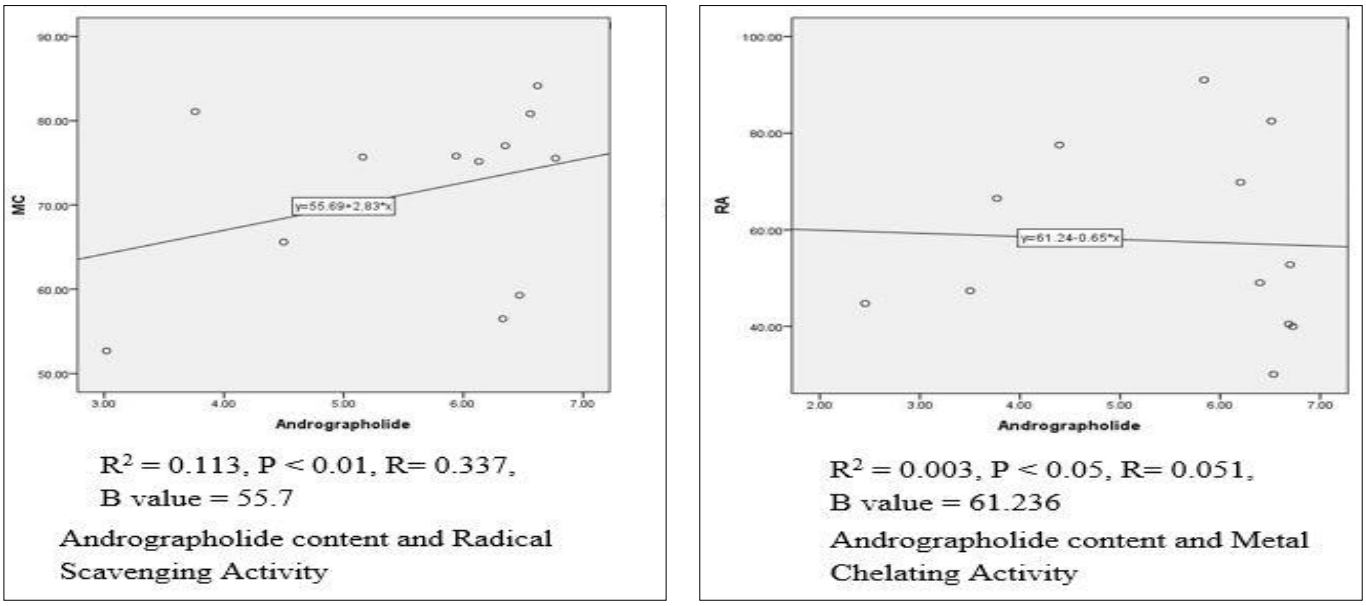


Fig. 6. Relationship Between andrographolide content, radical scavenging and metal chelating activity.

Linear regression shows that Andrographolide content positively correlates with Radical Scavenging Activity (y = 55.69 + 2.83x, R^2 = 0.113, P < 0.01, B = 55.7) but negatively correlates with Metal Chelating Activity (y = 61.24 - 0.65x, R^2 = 0.003, P < 0.05, B = 61.236). The B value represents the intercept, indicating baseline activity levels independent of Andrographolide content. The y-axis represents Radical Scavenging Activity (%) in the left graph and Metal Chelating Activity (%) in the right graph.

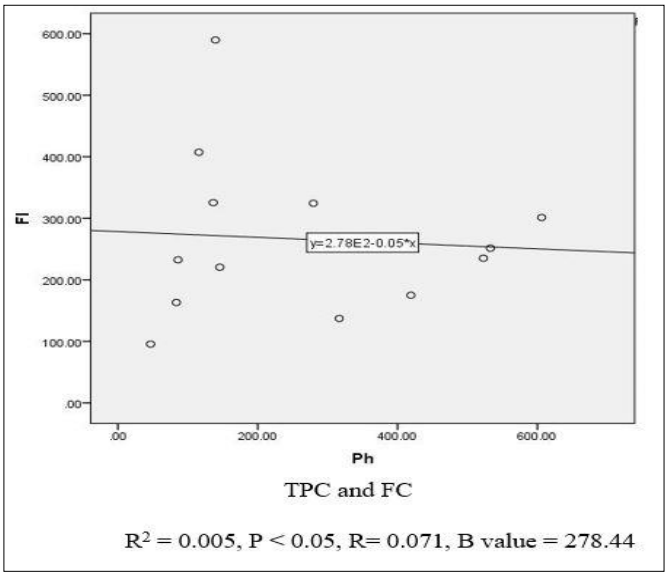


Fig. 7. Linear Regression Analysis of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC).

The regression equation (y = 278.44 - 0.05*x) indicates a weak negative correlation between TPC and TFC. The coefficient of determination (R^2 = 0.005) suggests that TPC explains only 0.5% of the variation in TFC, with a low correlation coefficient (R = 0.071). The B value (278.44) represents the intercept, indicating the estimated TFC when TPC is zero. The significance level (P < 0.05) suggests statistical relevance, but the weak correlation implies that TPC is not a strong predictor of TFC in this dataset.

In terms of productivity for andrographolide, phenols and flavonoids, Cd, As and Hg, imparted a net negative impact. The productivity was highest in control for andrographolide and TFC (Table 2). Notably, 50 µM Cd showed higher productivity for TPC (Table 2) indicating that *A. paniculata* cells may have tried to resist the deleterious effect of increasing concentration of Cd by producing more phenols which serve as antioxidants. A slight increase in the productivity for andrographolide with the increase in Cd concentration from 25 µM to 50 µM (Table 2) could be attributable to increase in TPC, which may have played a role in chelating Cd. Beyond 50 µM of Cd, productivity for TPC showed a downward trajectory (Table 2; concentrations higher that 50 µM were toxic. The cell suspension cultures were most susceptible to Hg (Table 2).

Conclusion

The study evaluated the impact of heavy metal elicitors on andrographolide and other secondary metabolites in *A. paniculata*. Compared to Cd and As, Hg was more sensitive to andrographolide production at higher concentrations. These observations are in tandem with the values seen in metal chelating activity, which corresponds to tolerance of the cells to a given concentration of the heavy metal

Table 2. Effect of Cd, As and Hg on the productivity (mg/L) of Andrographolide, Total Phenolic Content and Total Flavonoid Content of cell suspension cultures of *A. paniculata*

	Cd			As			Hg		
	TPC	TFC	Andro	TPC	TFC	Andro	TPC	TFC	Andro
Control	1.5±0.01b	0.7±0.02d	28.2±0.7a	1.5±0.01b	0.7±0.02d	28.2±0.7a	1.5±0.01b	0.7±0.02d	28.2±0.7a
25 µM	1.3±0.01c	0.5±0.01ef	17.8±0.01b	0.4±0.003ef	0.9±0.01c	18.9±0.3b	1.5±0.05b	0.72±0.02d	12.6±1.6c
50 µM	1.8±0.07a	0.8±0.01c	18.7±0.2b	0.3±0.003f	1.1±0.01b	18.1±0.12b	1.5±0.03b	0.7±0.02d	11.3±0.4c
75 µM	0.7±0.01d	0.9±0.03c	18.5±0.1b	0.2±0.03g	0.6±0.07de	16.9±1.73b	0.4±0.01e	0.6±0.01de	10.1±1.5c
100 µM	0.4±0.003ef	2.65±0.13a	18.1±0.12b	0.2±0.003g	0.4±0.01f	16.3±0.9b	0.1±0.001g	0.26±0.01g	6.8±0.8d

Data in the figure represent the mean value of the triplicates. Values after ± indicate the standard error.

Andrographolide productivity was of the order: Control > As25 µM > Cd50 µM > Cd 75 µM >As 50 µM > Cd 100 µM > Cd 25 µM. Total Flavonoid Productivity was of the order: Cd 100 µM > As 50 µM > As 25 µM > Hg 25 µM > Control. Total Phenol Productivity was of the order: Cd 50 µM > Hg 50 µM > Control > Hg 25 µM > Cd 25 µM

administered. 75 µM of As resulted in the production of highest amount of andrographolide content followed by 100 µM of Cd. The cell suspension cultures of *A. paniculata* showed higher sensitivity to Hg, which at higher concentrations imparted a negative effect on all the biochemicals constituents studied. While in control was most productive in terms of andrographolide production, a certain degree of tolerance was seen against Cd, whose toxicity seemed to be nullified by the corresponding increase in metal chelating activity and TPC. Therefore, owing to higher resistance against Cd and higher production of andrographolide, Cd could be employed as an elicitor in cell suspension cultures of *A. paniculata* at concentrations up to 50 µM for commercial scale production of andrographolide. Lower concentration of heavy metals could be explored for future studies.

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Authors' contributions

NA carried out the tissue culture aspects including development of the cell suspension culture, participated in the heavy metal treatment. NA carried out the phytochemical, physiological and quantification studies. NA and PN participated in the design of the study. NA performed the statistical analysis. Both NA and PN conceived of the study and participated in its design and coordination. Both authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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