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# Effect of CuO nanoparticles on polyphenols content and antioxidant activity in Ashwagandha (*Withania somnifera* L. Dunal)

**Oinam Surjit Singh, Naveen Chandra Pant, Lenin Laishram, Manisha Tewari, Rakesh Dhoundiyal, Karishma Joshi and CS Pandey**

**Abstract**
In the present investigation CuO nanoparticles significantly enhanced the polyphenols content in treated plants at different dates of sampling. Highest total phenol content i.e., 27.215±0.73 and 26.455±0.365 mg gallic acid equivalents/g extract, was observed 20 days after treatment in shoots and roots of treated plants, respectively. No significant difference ($p \leq 0.05$) in flavonoid content within shoots of treated plants was observed 15 and 20 DAT, respectively. Highest flavonoid content i.e., 23.076±5.128 mg quercetin equivalents/g extract was observed in roots of treated plants 20 days after treatment. Significantly higher tannin content was also observed in roots of treated plants20 DAT. Higher total antioxidant activity i.e., 55.797±1.924 and 56.811±3.012mg ascorbic acid equivalents/g extract was observed in shoots and roots of treated plants, respectively 20 days after treatment. Higher DPPH radical scavenging activity as observed by lower $IC_{50}$ values i.e., 336.234±5.187 and 473.88±8.834 µg/ml in shoots and roots of treated plants, respectively was observed 20 days after treatment. Total antioxidant activity in shoots showed a significant ($p \leq 0.05$) positive correlation with phenol and tannin content within the shoots i.e., 0.618 and 0.693, respectively. Total antioxidant activity in roots also showed significant positive correlation with total phenol, flavonoid and tannin content within roots i.e., 0.416, 0.683 and 0.891, respectively. DPPH radical scavenging activity within shoots (-0.888) and roots (-0.851) showed a significant negative correlation with total phenol and flavonoid content in shoots and roots, respectively. Thus the findings of the present investigation clearly shows, the elicitation effect of CuO nanoparticles in *Withania somnifera* L.

**Keywords:** antioxidants, phenol, flavonoids, DPPH

## Introduction
Ashwagandha (*Withania somnifera* L. Dunal) is an important medicinal crop cultivated in India. It is mentioned as an important drug in ancient Ayurvedic literature. The genus Withinia belongs to solanaceae and consist of 26 species. Two species of the genus, *Withania somnifera* L. Dunal and *W. coagluans* Dunal occurs in India (Alam *et al*., 2016) [3]. The species has been under domestication since long in Central India. In India the plant is grown throughout dry subtropical and temperate regions in the states of Madhya Pradesh, Rajasthan, Gujarat, Maharashtra, Andhra Pradesh, Uttar Pradesh, Haryana and Punjab extending to Himachal Pradesh and Jammu and Kashmir from plains upto a height of about 1700 m. It is mainly cultivated in MP, Punjab and some adjoining villages of Rajasthan (Shrivastava and Sahu, 2013) [18]. Alkaloids and withanolides are the major group of active principles, isolated and characterised from *Withania somnifera*. Withanolides are steroidal lactones and the leaves are of plant are characterised by the presence of various substituted steroidal lactones of the withanolide group. Withaferin-A is the major withanolides, isolated to which the curative, viz., antibacterial, anti-tumour and anti-inflammatory properties of leave are attributed to withanolide-D possessing marked anti-tumour property (Devi *et al*., 1993) [7] against Sarcoma and Ehrlich ascites carcinoma and withanolide-E possessing immune suppressive activity are the major withanolides isolated from the plant (Jain *et al*., 2012) [9].
Ashwagandha is widely used in Indian systems of medicine and Homeopathy to cure diseases like leprosy, nervous disorders, intestinal infections and rheumatism. The berries and seeds are diuretic and are also used for treating chest complaints. Studies have shown that ashwagandha is effective in the treatment of osteoarthritis, inflammation, stroke, and tardive dyskinesia. Ayurvedic practitioners have used the roots of this plant for centuries with success to treat health conditions (Umadevi *et al*., 2012) [21]. Ashwagandha is used to calm the mind, relieve weakness and nervous exhaustion, build sexual energy and promote healthy sleep. The herb is termed as rasayan in Ayurvedic practice, which means it acts as a tonic for vitality and

longetivity. It is also classified as an adaptogen. The preventive effects of plant polyphenols and their use in treating diseases are deduced from the epidemiological data as well as in vitro and *in vivo* studies (Art and Hollamn, 2005) [4]. Many research studies have demonstrated that medicinal plants, fruits, and vegetables contain various phytochemicals with antioxidant activity, which are responsible for their beneficial health effects (Scalbert *et al*., 2005) [15]. Human body can be protected from these harmful compounds by its intrinsic enzymatic system, scavengers and antioxidants (Akinpelu *et al*., 2010) [2]. The antioxidants form an intricate network and are capable of preventing oxidative processes by inhibiting the initiation or propagation of an oxidative chain reaction and thereby can prevent many oxidative-stress related diseases (Kumaran and Karunakaran, 2007) [11].

Plants are an important source of bioactive molecules for drug discovery. Isolated bioactive molecules serves as starting material for laboratory synthesis of drugs as well as a model for the production of biologically active compound. Elicitation of secondary metabolites is of great significance to enhance the production of secondary metabolites in plants. An elicitor activates a signal-transduction cascade, which mediates the expression of genes related to the biosynthesis of secondary metabolites. The mechanism of elicitation is vastly complex because of thousands of intertwined events. Additionally, it fluctuates with the origin, specificity and concentration of elicitors, growth stage, nutritional status of plants and physiochemical environment of the interaction etc. Nanoparticles are currently emerging as new class of elicitors. A few studies on the induction of secondary metabolites in response to nanoparticle treatment provide insights in the exploitation of these submicron size particles as elicitors of secondary metabolites (Shakya *et al*., 2017) [16]. Phytochemical processing of raw plant materials is essentially required to optimize the concentration of known constituents and also to maintain their activities (Aziz *et al*., 2003) [5]. Extraction is an important step in the itinerary of phytochemical processing for the discovery of bioactive constituents from plant material. The present investigation was thus undertaken to investigate the effect of copper nano particles on various phytochemicals and the antioxidant activity of shoot and root extracts of *Withania somnifera*.

## Material and Methods

### Chemicals and reagents

The compounds 1, 1-diphenyl-2-picrylhydrazyl (DPPH), quercetin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade. CuO nano-particles used in the present investigation were prepared and provided by Dr. Karishma Joshi, Department of Biochemistry, Dolphin (P.G.) Institute of Biomedical and Natural Sciences, Dehra Dun 248007, Uttarakhand, India.

### Samples

The plants of *Withania somnifera* L. were grown under control condition. Half of the plants were treated with 1ppm copper nano particle solution and the remaining half used as control (untreated) plants. 15 and 20 days after treatment (DAT), the shoots and roots of plants, each from treated and untreated (control) were shade dried separately at $45^0C \pm 5^0C$ for a week. The dried shoots and roots were then powdered using Pestle and Mortar. The powdered samples were dissolved in methanol and incubated at room temperature for 24 hours. The solution was then filtered using WhatmanNo.1 filter paper (thrice). The combined methanolic extract was dried using rotatory flask evaporator. The dried extract so obtained was redissolved in methanol to prepare respective stock solutions (10 mg /ml). The extracts were stored at -20 $^0C$ for experimental use. Methanolic shoot and root extracts were used for quantification of polyphenols and antioxidant activity.

### Determination of Polyphenols and Antioxidant activity

### Total phenol content

The total phenolics content of the extract was estimated according to the method of Folin-Ciocalteu described by Swain and Hills (1959) [19]. 100 µl of the plant extract was used to determine total phenol content. The extract was diluted 10 folds by adding 900 µl methanol. 0.5ml Folin-Ciocalteu reagent was added and mixed well. After 6 min, 1ml of saturated $Na_2CO_3$ was added. The total volume was made to 7.5 ml using distilled water and the reaction mixture was allowed to settle down for at least 2-3 hours. After 2-3 hours, the absorbance was measured at 680 nm against a blank solution. The total phenol content was expressed as mg gallic acid equivalents (GAE) /g extract, using a calibration curve of gallic acid (200-1000 µg).

### Determination of total flavonoid content

The total flavonoids contents were estimated by Aluminium chloride colorimetric assay explained by Kim *et al*., 2003 [10]. 100 µl of the plant extract was diluted with 900 µl in a test tube. The volume was made to 4 ml using distilled water. In this 0.3ml of 5% $Na_2NO_3$ was added and kept for 5 minutes. After few minute 0.3 ml of $AlCl_3$ and 2 ml of 1M NaOH was added. The reaction mixture was allowed to stand for few minutes and the absorbance was measured at 510 nm against a blank solution. The amount of flavonoids in the extracts was expressed as mg equivalents of quercetin equivalents (QE)/gram extract, using a calibration curve of quercetin (20-100 µg).

### Determination of total tannin content

The total tannins content were determined using Folin-Denis method (Polshettiwar *et al*., 2007). To 100 µl of the extract, 900 µl of methanol was added. The volume was made to 7.5 ml using distilled water. To this, 0.5 ml of Folin-Denis reagent was added along with 1 ml $Na_2CO_3$. The total volume was adjusted to 10 ml using distilled water. The reaction mixture was kept for 2-3 hours and respective absorbance was measured at 680 nm against a blank solution. The amount of tannins was expressed as mg tannic acid equivalent/gram extract, using a calibration curve of tannic acid (20-100 µg).

### Determination of total antioxidant capacity (Phosphomolybdate assay)

The total antioxidant capacity of the methanol fraction was determined by Phosphomolybdate method (Prieto *et al*., 1999) using ascorbic acid as a standard. An aliquot of 100 µlof sample solution was mixed with 400 µl methanol and 1ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) making total volume 1.5 ml. Sample tubes were capped and incubated in a water bath at 95 ºC for 90 min. After cooling absorbance of the reaction mixture was measured at 680 nm against a blank. The total antioxidant capacity was expressed as ascorbic acid equivalents/g extract, using a calibration curve ascorbic acid (20-100 µg).

**DPPH radical scavenging activity**

DPPH radical scavenging activity was measured using the method described by Shih *et al*., (2010) [17]. This assay is based on the determination of concentration of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is a stable (in powder form) free radical with red colour which turns yellow when scavenged (Braca *et al*., 2001) [6]. The DPPH assay uses this character to show free radical scavenging activity. The aliquot of the extract in varying concentration (ranging from 20-100 µg/ml) were mixed with 2 ml DPPH (0.1 mM in methanol). After incubating for 30 minutes at 37ºC the absorbance was recorded at 520 nm with a colorimeter. Antioxidants react with DPPH and reduce it to DPPH-H and as a consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. The following formula has been used to determine the percentage of scavenging activity:

$$\%\ DPPH\ radical\ Scavenging = \left[1 - \left(\frac{Sample_{Abs}}{Control_{Abs}}\right)\right] \times 100$$

***Statistical analysis***

Analysis of variance (ANOVA) and Duncan LSD post hoc test were carried out on the values obtained in the experiment. Correlation analysis (bivariate) was also carried out to determine the relationship between polyphenol (i.e., total phenol, flavonoids and tannins) content and antioxidant activity determined by total antioxidant activity assay (Phosphomolybdate method) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity present in shoots and roots extracts of control and treated plants of *Withania somnifera* L. under investigation. The software IBM SPSS Statistics 20 (IBM Corporation) and SigmaPlot for Windows 11.0 (Systat Software, Inc.) were used to perform statistical analysis and graphing respectively. Results are expressed as mean ± SEm (n=3). A statistical difference at $p \leq 0.05$ was considered to be significant.

## Results

### Polyphenol content

**Total Phenol Content**

Total phenol content was expressed as mg gallic acid equivalents/g extract (Figure 1). The phenol content in shoots varied from 28.164±0.548 to 26.898±0.913 mg gallic acid equivalents/g extract in control plants from 15 to 20 days, respectively. In treated plants phenol content in shoots varied from 21.202±0.182 mg gallic acid equivalents/g extract, 15 DAT to 27.215±0.73 mg gallic acid equivalents/g extract, 20 DAT. In roots of control plants the phenol content varied from 26.772±0.182 mg gallic acid equivalents/g extract, 15 DAT to 26.139±0.182 mg gallic acid equivalents/g extract 20 DAT. In treated plants phenol content in roots varied from 25.348±0.091mg gallic acid equivalents/g extract, 15 DAT to 26.455±0.365 mg gallic acid equivalents/g extract, 20 DAT.

**Total flavonoid Content**

Total flavonoid content was expressed as mg quercetin equivalents/g extract (Figure 2). The flavonoid content in shoots varied from 16.153±0.00 to 14.615±2.220 mg quercetin equivalents/g extract in control plants from 15 to 20 days, respectively. In treated plants no significant difference ($p \leq 0.05$) in flavonoid content within shoots was observed from 15 to 20 DAT. In roots of control plants the flavonoid content varied from 11.538±2.22 mg quercetin equivalents/g extract, 15 DAT to 15.384±4.441 mg quercetin equivalents/g extract 20 DAT. In treated plants flavonoid content in roots varied from 19.23±2.22 mg quercetin equivalents/g extract, 15 DAT to 23.076±5.128 mg quercetin equivalents/g extract, 20 DAT.

**Total Tannin Content**

Tannin content was expressed as mg tannic acid equivalents/g extract (Figure 3). The tannin content in shoots varied from 4.029±1.292 to 6.268±0.861 mg tannic acid equivalents/g extract in control plants from 15 to 20 days, respectively. In treated plants tannin content in shoots varied from 2.835±0.861 mg tannic acid equivalents/g extract, 15 DAT to 3.283±0.861 mg tannic acid equivalents/g extract, 20 DAT. In roots of control plants the tannin content varied from 3.134±1.292 mg tannic acid equivalents/g extract, 15 DAT to 2.641±1.292 mg tannic acid equivalents/g extract 20 DAT. In treated plants tannin content in roots varied from 3.134±0.43 mg tannic acid equivalents/g extract, 15 DAT to 5.373±0.861 mg tannic acid equivalents/g extract, 20 DAT.

### Antioxidant Activity

**Total Antioxidant Activity**

Total antioxidant activity was expressed as mg ascorbic acid equivalents/g extract (Table 1). The total antioxidant activity in shoots varied from 44.347±5.522 to 58.55±3.012 mg ascorbic acid equivalents/g extract in control plants from 15 to 20 days, respectively. In treated plants total antioxidant activity in shoots varied from 39.565±1.087 mg ascorbic acid equivalents/g extract, 15 DAT to 55.797±1.924 mg ascorbic acid equivalents/g extract, 20 DAT. In roots of control plants the total antioxidant activity varied from 52.753±2.348 mg ascorbic acid equivalents/g extract, 15 DAT to 49.710±0.251 mg ascorbic acid equivalents/g extract 20 DAT. In treated plants total antioxidant activity in roots varied from 52.173±0.334 mg ascorbic acid equivalents/g extract, 15 DAT to 56.811±3.012 mg ascorbic acid equivalents/g extract, 20 DAT.

**DPPH radical scavenging activity**

DPPH radical scavenging activity was used as a measure of antioxidant activity in methanolic shoot and root extract of treated and untreated (control) plants. DPPH radical scavenging increased with increase in concentration of the extracts in both treated and untreated plants (Figure 4 A & B). The scavenging activity was expressed as $IC_{50}$ values of the extracts, i.e., the inhibitory concentration which was able to scavenge 50% of DPPH radicals (Table 1). The $IC_{50}$ values in shoots varied from 395.335±3.093 to 361.688±1.597 µg/ml in control plants from 15 to 20 days, respectively. In treated plants $IC_{50}$ values in shoots varied from 512.713±2.002 µg/ml, 15 DAT to 336.234±5.187 µg/ml, 20 DAT. In roots of control plants the $IC_{50}$ values varied from 586.816±21.921 µg/ml, 15 DAT to 522.936±11.817 µg/ml, 20 DAT. In treated plants $IC_{50}$ values in roots varied from 540.822±17.318 µg/ml, 15 DAT to 473.88±8.834 µg/ml, 20 DAT.

## Discussion

### Polyphenol content

Plant polyphenols are known to possess significant free radical scavenging. Flavonoid and other phenolic compounds of plant origin have been reported as free radical scavengers and many pharmacological effects shown by plant based formulations are due to presence of polyphenols. Present

investigation clearly shows the elicitation effect of CuO nano particles on polyphenols content in shoots and roots of treated plants. Both shoots and roots of *Withania* showed significantly ($p \leq 0.05$) higher phenol and flavonoid content at 20 days after treatment (DAT). The total phenol content in shoots and roots of treated plants at 20 DAT differed significantly w.r.t control and treated plants at 15 and 20 DAT, respectively. Similarly significantly higher flavonoid content was observed in shoots and roots of treated plants at 20 DAT, which differed significantly ($p \leq 0.05$) w.r.t control and treated plants at 15 DAT. Higher tannin content was observed in roots of treated plants which differed significantly ($p \leq 0.05$) w.r.t tannin content in roots of control and treated plants at 15 and 20 DAT, respectively. The total phenol content in shoots showed a significant ($p \leq 0.01$) positive correlation with total phenol content in roots (0.949). It also showed a positive correlation with flavonoid and tannin content in shoots i.e., 0.328 and 0.487, respectively (Figure 5 A & B). The total phenol content in roots showed a positive correlation with flavonoid content in shoots and tannin content in roots i.e., 0.485 and 0.352, respectively (Figure 6 A & B). Flavonoid content in roots and shoots also showed a positive correlation (0.238). It also showed a significant ($p \leq 0.01$) positive correlation with tannin content in roots (0.812).

Recently dietary polyphenols have received attention of scientists and consumers due to their potential medicinal properties. Experimental evidences both *in vitro* and *in vivo* over the years have provided definite proofs which strongly supports for a possible role for dietary polyphenols in the prevention and cure of various degenerative diseases, particularly cancers, cardiovascular diseases and neurodegenerative diseases (Tsao, 2010) [20]. Polyphenols are strong antioxidants that complements defence against oxidative stress caused by reactive oxygen species (ROS) associated with various diseases. Nanoparticle mediated elicitation of secondary metabolites has also been observed by Ghanati *et al*., 2014 [8]. The present investigation clearly shows the possible effect of CuO nano particles in eliciting polyphenols in *Withania somnifera* and it may be used to enhance polyphenols content in the plant. Conclusive evidences may only be there if more investigations are pursued so as to understand the possible mechanism of increase in polyphenols within the plant.

**Antioxidant activity**

The total antioxidant potential serves as a relevant tool for investigating the relationship between dietary antioxidants and pathologies induced by the oxidative stress associated with various diseases. The total antioxidant activity measured by Phosphomolybdate method is a sensitive method to determine total antioxidant potential of natural antioxidants. Significantly higher ($p \leq 0.05$) total antioxidant activity was observed in treated plants at 20 DAT w.r.t treated and control plants at 15 DAT. Total antioxidant activity in shoots of control and treated plants showed a significant positive correlation ($p \leq 0.05$) with phenol and tannin content in the shoots i.e., 0.618 and 0.693, respectively. Total antioxidant activity in roots of control and treated plants also showed a positive correlation with phenol, flavonoid and tannin content within the roots i.e., 0.416, 0.683 and 0.891, respectively. Nanoparticle mediated elicitation of secondary metabolites has also been observed by Ghanati *et al*., 2014 [8]. The results clearly shows that the variation in total antioxidant activity within CuO nanoparticle treated plants are due to changes in the intrinsic levels of polyphenols (phenol, flavonoid and tannins) within the plants.

DPPH scavenging capacity test is an important free radical scavenging assay and may be effectively used to identify primary antioxidants within plants, which can donate the hydrogen to scavenge free radicals (Ajila *et al*., 2007; Wang *et al*., 2008; Nurliyana *et al*., 2010) [1, 22, 12]. Dietary polyphenols are one of the potential candidates to serve as primary antioxidants in various plant based formulations. The DPPH radical scavenging activity within shoots and roots of control and treated plants of *Withania somnifera* L. differed significantly ($p \leq 0.05$). Higher radical scavenging was observed at 20 DAT in both shoots and roots of treated plants. DPPH radical scavenging in treated plants at 20 DAT differed significantly ($p \leq 0.05$) w.r.t control plant at 20 DAT and control, treated plants at 15 DAT. DPPH radical scavenging within shoots showed a significant negative correlation ($p \leq 0.01$) with total phenol content within the shoots (-0.888), clearly showing that even at lower concentration the shoots extracts were able to scavenge DPPH free radicals, as observed by lower $IC_{50}$ values of the extracts. Similarly within roots DPPH radical scavenging activity showed a significant negative correlation ($p \leq 0.01$) with total flavonoid content within the roots (-0.851), clearly showing that even at lower concentration the roots extracts were able to scavenge DPPH free radicals, as observed by lower $IC_{50}$ values of the extracts. Nanoparticle mediated elicitation of secondary metabolites has also been observed by Ghanati *et al*., 2014 [8]. The results clearly shows that the variation in DPPH radical scavenging activity within CuO nanoparticle treated plants are due to changes in the intrinsic levels of polyphenols (phenol and flavonoid) within the plants.

The higher free radical scavenging potential as observed by higher total antioxidant activity and DPPH scavenging activity may be associated with higher levels of polyphenols as observed in treated plants under the present investigation. These natural antioxidants are multifunctional and they might contribute significantly higher antioxidant activity in CuO nanoparticle treated plants of *Withania somnifera* L. as observed in the present investigation.

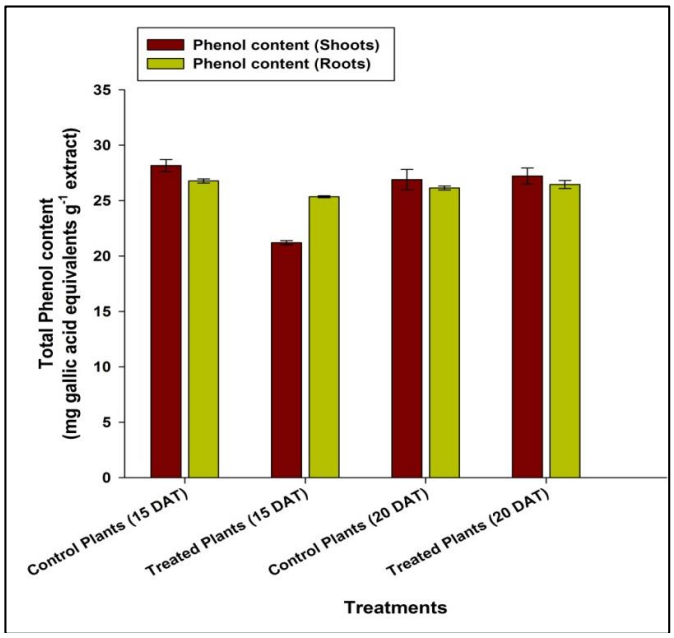


**Fig 1:** Total Phenol content in (Shoots and Roots) Control and Treated plants of *Withania somifera*. Data shown below are mean value ± standard error (n=3)

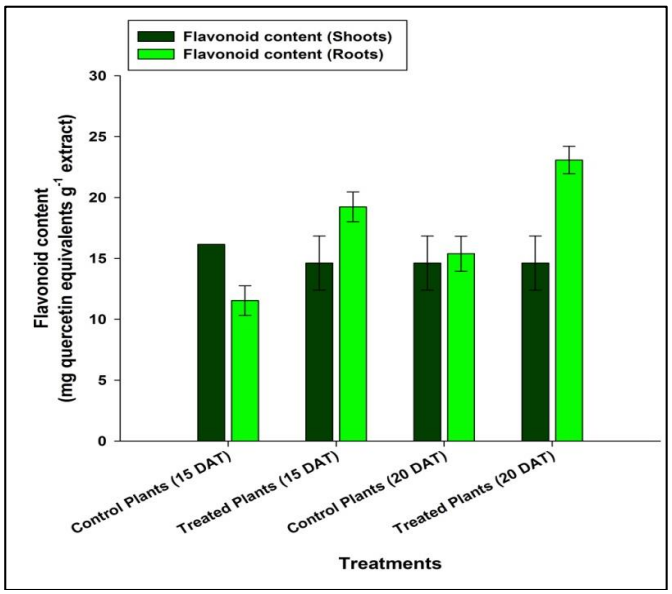


**Fig 2:** Flavonoid content in (Shoots and Roots) Control and Treated plants of *Withania somifera*. Data shown below are mean value ± standard error (n=3)

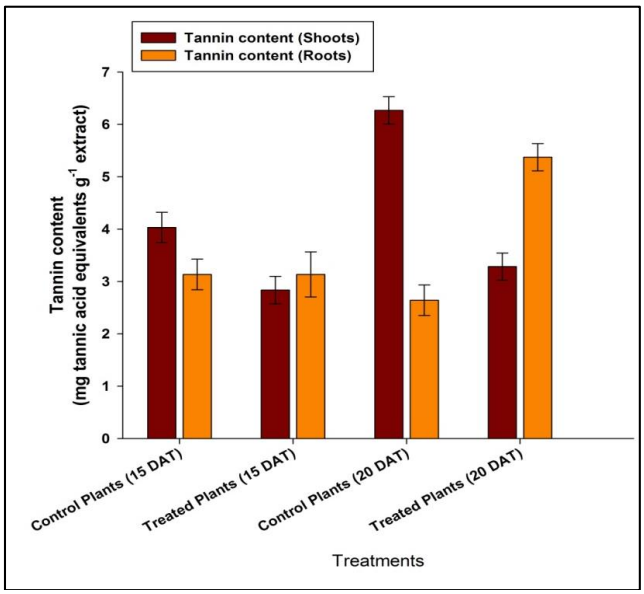


**Fig 3:** Tannin content in (Shoots and Roots) Control and Treated plants of *Withania somifera*. Data shown below are mean value ± standard error (n=3)

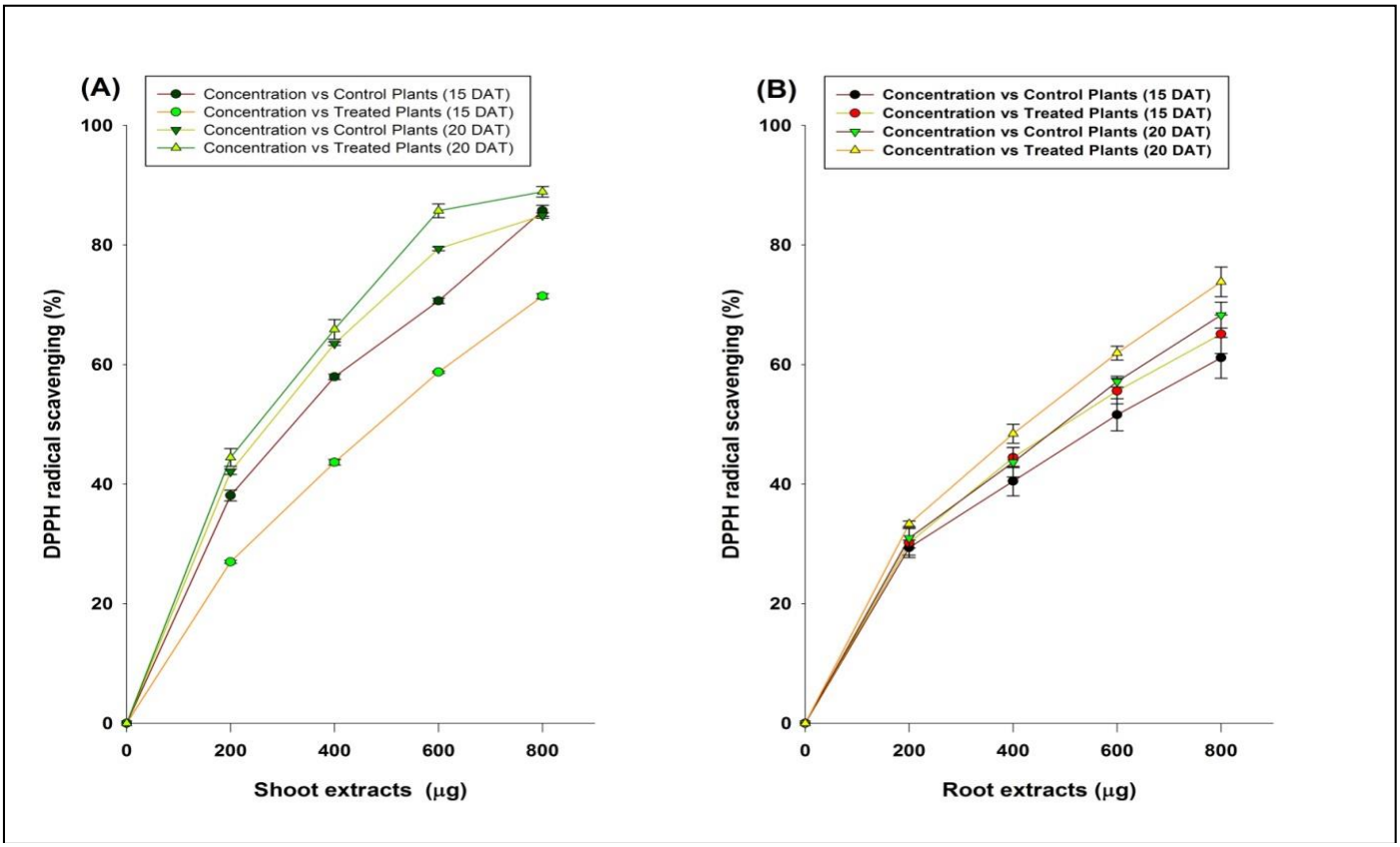


**Fig 4:** DPPH radical scavenging in (A) Shoots and (B) Roots of Control and Treated plants of *Withania somifera*. Data shown below are mean value ± standard error (n=3)

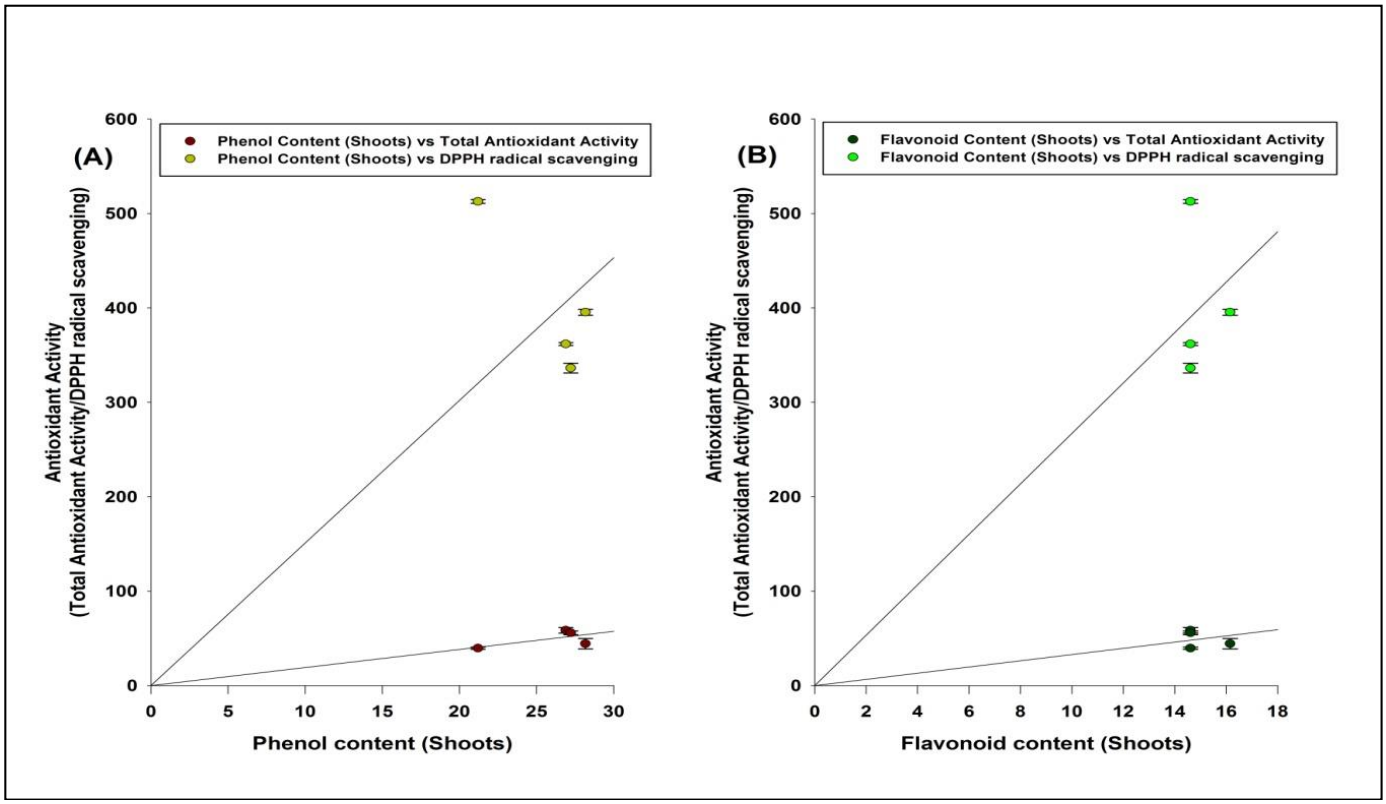


**Fig 5:** Correlation analysis (A): Between phenol content and antioxidant activity in shoots of control and treated plants of *Withania somifera*. (B): Between flavonoid content and antioxidant activity in shoots of control and treated plants of *Withania somifera*

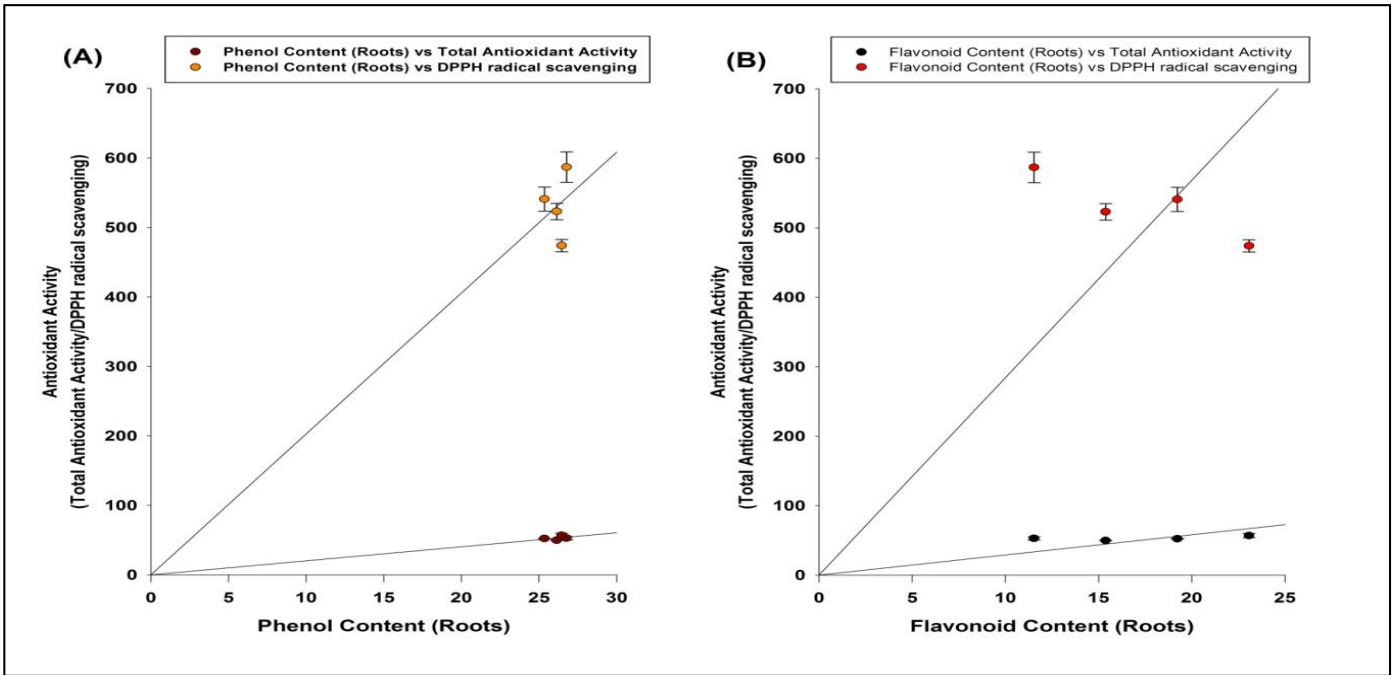


**Fig 6:** Correlation analysis (A): Between phenol content and antioxidant activity in roots of control and treated plants of *Withania somifera*. (B): Between flavonoid content and antioxidant activity in roots of control and treated plants of *Withania somifera*

**Table 1:** Total antioxidant activity (mg ascorbic acid equivalent/g extract) and DPPH radical scavenging ($IC_{50}$) in shoots and roots of control and treated plants of *Withania somifera*. Data shown below are mean value ± standard error (n=3)

| Treatments | Total antioxidant capacity in Shoots (mg ascorbic acid equivalent) | Total antioxidant capacity in Roots (mg ascorbic acid equivalent) | DPPH radical scavenging in Shoots ($IC_{50}$) | DPPH radical scavenging in Roots ($IC_{50}$) |
|---|---|---|---|---|
| Control Plants (15 DAT) | $44.347 \pm 5.522^{b}$ | $52.753 \pm 2.348^{b}$ | $395.335 \pm 3.093^{c}$ | $586.816 \pm 21.921^{c}$ |
| Treated Plants (15 DAT) | $39.565 \pm 1.087^{b}$ | $52.173 \pm 0.334^{b}$ | $512.713 \pm 2.002^{d}$ | $540.822 \pm 17.318^{bc}$ |
| Control Plants (20 DAT) | $58.550 \pm 3.012^{a}$ | $49.710 \pm 0.251^{b}$ | $361.688 \pm 1.597^{b}$ | $522.936 \pm 11.817^{ab}$ |
| Treated Plants (20 DAT) | $55.797 \pm 1.924^{a}$ | $56.811 \pm 3.012^{a}$ | $336.234 \pm 5.187^{a}$ | $473.880 \pm 8.834^{a}$ |

***Note:** The values with same superscript are not significantly different at ($P \leq 0.05$) according to Duncan LSD post hoc analysis.

## Conclusion

The results clearly showed that CuO nanoparticle treated plants showed significantly higher polyphenols and corresponding antioxidant activity at 20 DAT. The phenol and flavonoid content within the shoots and roots of treated plants were positively correlated with total antioxidant activity and DPPH scavenging activity. Thus the present investigation may be helpful in understanding the ability of Cuo nanoparticles to elicit polyphenols within *Withania somnifera* L. and it may serve as a basis for future investigation in understanding the mechanism of polyphenols synthesis and elicitation within the plants.

## Conflict of interest

We the authors declare that we have no conflict of interest.

## Acknowledgements

The infrastructure provided by Department of Agriculture, Dolphin (P.G.) Institute of Biomedical and Natural Sciences, Dehra Dun. Uttarakhand and Cuo nanoparticles provided by Dr. Karishma Joshi (Department of Biochemistry, Dolphin (P.G.) Institute of Biomedical and Natural Sciences, Dehra Dun Uttarakhand, India) is duly acknowledged.

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