



Comparative Evaluation of Phytochemicals and Antioxidant Potential of *Cleome viscosa* and *Trichodesma indicum*

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ABSTRACT

Cleome viscosa and *Trichodesma indicum* are well-known medicinal plants. Their leaves, fruits and root extracts were obtained and investigated for their total phenols, flavonoids and alkaloids contents respectively. The enzymic (catalase, peroxidase, polyphenol oxidase, superoxide dismutase and glutathione-S-transferase) and non-enzymic (ascorbic acid, α -tocopherol, reduced glutathione and flavonoids) antioxidant components were determined by using standard protocols. It was found that all the phytochemicals analysed were present in both the fruit and root of *Cleome viscosa* and *Trichodesma indicum*. The leaves of *Cleome viscosa* did not show the presence of alkaloids and the leaves of *Trichodesma indicum* did not found to contain the flavonoids. The activity of catalase was higher in the fruits (118.9 ± 5.6 U/g) of *Cleome viscosa* and leaves (117 ± 4.1 U/g) of *Trichodesma indicum*. In the case of peroxidase and glutathione-S-transferase the maximum result was shown by both the fruits and roots of *Cleome viscosa* and *Trichodesma indicum*. A significant non-enzymic antioxidant activity was found in both the plants. From the present study it is evident that the leaf, fruit and root extracts of *Cleome viscosa* and *Trichodesma indicum* possess good antioxidant activity. Hence the plant can be further explored to be used as a potent therapeutic agent for free radical related pathological damage.

Keywords: Antioxidants, *Cleome viscosa*, Phytochemicals, *Trichodesma indicum*.

INTRODUCTION

Medicinal plants play a key role in the human health care. The traditional medicine refers to a broad range of ancient natural health care practices including folk/tribal practices as well as Ayurveda, Siddha, Amchi and Unani. These medical practices originated from time immemorial and developed gradually, to a large extent, by relying or based on practical experiences without significant references to modern scientific principles. These practices incorporated ancient beliefs.¹ The medicinal value of these plants lies in some chemical substances called phytochemicals that produce a definite physiological action on the human body.² Investigations into the chemical and biological activities of plants during the past two centuries have yielded compounds for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents.³

Phytochemicals are bioactive non-nutritive compounds found in plants that work with nutrients and dietary fiber to protect against diseases. Many phytochemicals such as alkaloids, carotenoids and flavonoids have antioxidant activity and reduce the risk of many diseases.⁴ To date, many plants have been claimed to pose beneficial health effects such as antioxidant properties.⁵ Antioxidants, the free radical scavengers can prevent pathological conditions of human body namely ischemia, anemia, asthma, arthritis, inflammation, neurodegeneration and aging process. Medicinal plants are the basis of the modern pharmaceuticals used today for various ailments, as it has a good source of natural antioxidants for medicinal use and related to radical mechanism.⁶

Cleome viscosa L., (Cleomaceae) is a widely distributed sticky herb with yellow flowers and long slender pods containing seeds, which are similar to those of mustard (Hindi), Hurhuria (Bengali), Nayikkadugu (Tamil) in South Asian folk medicine, found throughout the larger part of Indian subcontinent, often in waste places.⁷

Trichodesma indicum (Boraginaceae) commonly known as Adhapushpi. It is a perennial medicinal herb distributed in tropical and subtropical Asia, Africa and Australia. It is a multidrug plant used to reduce or cure inflammation, pain, osteoarthritis and conjunctivitis. The plant is used for expulsion of dead fetus abortion, inhibition of diarrhoea, reduction of sulfur dioxide-induced cough reflex in mice, as brain tonic and also to treat breast cancer.⁸

The present study deals with the analyses of phytochemicals and antioxidants in leaves, fruits and roots of *Cleome viscosa* and *Trichodesma indicum*.

MATERIALS AND METHODS

Collection of plant material

The plant samples were collected from Erode district and were identified by the Botanical Survey of India, Coimbatore. Fresh leaves, fruits and roots of *Cleome viscosa* and *Trichodesma indicum* (Plate I and II) were collected and cleaned to remove adhering dust particles, washed under running tap water and gently blotted dry between folds of tissue paper. They were then dried at room temperature in shade away from direct sunlight, powdered and stored at room temperature until use.

Chemicals

Hydrogen peroxide, potassium hydrogen phosphate, potassium hydrogen buffer, ferrous chloride, ferric chloride, ferric cyanide, hydrogen peroxide, ferrous ammonium sulfate, ethylene diamine tetra-acetic acid (EDTA) disodium salt, N- (1-naphthyl) ethylene diamine dihydrochloride, riboflavin, sodium nitroprusside, pyrogallol, potassium persulfate were obtained from Himedia and Merck. All other chemicals and solvents used were of analytical grade.

Preliminary phytochemical screening

The active constituents such as alkaloids, polyphenols and flavonoids have been identified by various preliminary tests.

Detection of alkaloids

Mayer's test

A fraction of the extract was treated with Mayer's reagent (1.36g of mercuric chloride and 5g of potassium iodide in 100ml of distilled water) and observed for the formation of cream coloured precipitate.

Dragendroff's test

An aliquot of the extract was tested with Dragendroff's reagent (Solution A: Bismuth nitrate (0.17g) in glacial acetic acid (2ml) and distilled water (8ml) Solution B: Potassium iodide (4gm) in glacial acetic acid (10ml) in water (20ml), Mix solutions A and B and dilute to 100ml with distilled water) and observed for the formation of reddish orange precipitate.

Wagner's test

A fraction of the extract was treated with Wagner's reagent (1.2g of iodine and 2g of potassium iodide in 100ml of distilled water) and observed for the formation of reddish brown coloured precipitate.

Detection of phenolics

Ferric chloride test

To a fraction of the extract, 5% FeCl₃ solution was added and observed for the formation of deep blue colour.

Lead acetate test

A fraction of the extract was treated with 10% lead acetate solution and observed for the formation of white precipitate.

Detection of flavonoids

Aqueous NaOH test

To a fraction of the extract, a drop of 1N aqueous NaOH solution was added and observed for the formation of yellow orange colouration.

Sulphuric acid test

A fraction of the extract was treated with concentrated H₂SO₄ and observed for the formation of orange colour.

Schinodo's test

A fraction of the extract was treated with a piece of magnesium turnings followed by a few drops of concentrated hydrochloric acid, heated slightly and observed for the formation of dark pink colour.

Determination of the enzymic and non-enzymic antioxidants

Determination of catalase activity

Homogenized the sample in a blender with M/15 phosphate buffer (pH 7.0) at 4°C and centrifuged. Stirred the sediment with cold M/15 phosphate buffer (pH 7.0), allowed to stand in the cold with occasional shaking and then repeated the extraction once or twice. The combined supernatants were used for the assay. Read against a control cuvette containing the enzyme solution as in the experimental cuvette, but containing H₂O₂ free M/15 phosphate buffer (pH 7.0). Pipetted into the experimental cuvette 3.0 ml of H₂O₂-phosphate buffer (0.16 ml of H₂O₂ made upto 100 ml with M/15 phosphate buffer, pH 7.0). Mixed in 0.01- 0.04 ml sample and noted the time it required for a decrease in absorbance from 0.45 to 0.4. Calculated the concentration of H₂O₂ using the extinction coefficient 0.036 per μ mole per ml.

Estimation of peroxidase activity

Macerated one gram of the sample with 5 ml (w/v) 0.1M phosphate buffer (pH 6.5) in a homogenizer. Centrifuged the homogenate at 300 g for 15 min. Used the supernatant as the enzyme source. All the procedures were carried out at 0-5°C. Pipetted out 3ml of 0.05 M pyrogallol solution and 0.5 to 1.0ml of enzyme extract in a test tube. Adjusted the spectrophotometer to read '0' at 400 nm. Added 0.5ml of 1% hydrogen peroxide in the test cuvette. Recorded the change in the absorbance every 30 seconds up to 3 minutes.

Determination of superoxide dismutase activity

The incubation medium contained in a final volume of 3.0 ml, 50 mM potassium phosphate buffer (pH 7.8), 45 μ M methionine, 5.3 μ M riboflavin, 84 μ M NBT and 20 μ M KCN. The tubes were placed in an aluminium foil-lined box maintained at 25°C and equipped with 15W fluorescent lamps. Reduced NBT was measured spectrophotometrically at 600 nm after exposure to light for 10 minutes. The maximum reduction was evaluated in the absence of the enzyme. One unit of enzyme activity was defined as the amount of enzyme causing 50 per cent inhibition of the reduction of NBT.

Estimation of polyphenol oxidase

Grind about 5g of the plant sample and made upto 20ml with the medium containing 50mM Tris-HCl (pH 7.2), 0.4M sorbitol and 10mM NaCl. Centrifuge the homogenate at 2000rpm for 10 minutes and used the supernatant for the assay. Added 2.5ml of 0.2M phosphate buffer (pH 6.5), 0.3ml of catechol solution (0.01 M) into the cuvette and set the spectrophotometer

at 495nm. Added the enzyme extract (0.2ml) and started recording the change in absorbance for every 30 seconds upto 5 minutes.

Estimation of glutathione-s-transferase activity

GST conjugates with GSH and CDNB and the extent of conjugation causes a proportionate change in the absorption at 340nm. The sample was homogenized with Tris-HCl buffer (pH7.2). The homogenated sample was centrifuged at 4°C for 30 minutes at 8500rpm. The supernatant was used as the enzyme source. The estimation was done at 25°C under condition giving activities linear with respects to incubation time and protein concentration for at least 3 minutes. The enzyme activity was determined by monitoring the change in absorbance at 340nm in a spectrophotometer. 0.1ml of both substrates GSH and CDNB was taken in 0.1M phosphate buffer (pH 6.5) at room temperature to make a volume of 2.9ml. The reaction was started by the addition of 0.1ml of sample to this mixture; the readings were recorded against distilled water blank for a minimum of three minutes. The complete assay mixture without the sample served as the control to monitor non-specific binding of the substrate. It was taken to ensure that final concentration of ethanol in the mixture was always less than 4%. GST activity was calculated using the extinction coefficient of the product formed and the values have been expressed as nmoles and CDNB conjugated/minutes/g sample.

Estimation of ascorbic acid

One gram of the sample was homogenized in 10 ml of 4 per cent TCA. Centrifuged at 2000 rpm for 10 minutes. The supernatants obtained were treated with a pinch of activated charcoal. Shaken well and kept for 10 minutes. Centrifuged and 0.5 and 1.0 ml aliquots of this supernatant were taken for the assay. The assay volumes were made up to 2.0 ml with 4 per cent TCA. The working standard solution containing 20-100 µg of ascorbate were pipetted out, the volumes were also made up to 2.0 ml with 4 per cent TCA. Added 0.5 ml of 2 per cent DNPH (2,4-dinitrophenyl hydrazine in 9 N H₂SO₄) reagent to all the tubes, followed by 2 drops of 10 per cent thiourea solution. Incubated at 37°C for 3 hrs. The osazones formed were dissolved in 2.5 ml of 85 per cent sulphuric acid, in cold, drop by drop, with no appreciable rise in temperature. To the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid. After incubation for 30 minutes at room temperature, the absorbance was read spectrophotometrically at 540 nm. Calculated the content of ascorbic acid in the sample using the standard graph.

Estimation of α- tocopherol

The samples were weighed (2.5 g) and added 50 ml of 0.1N sulphuric acid slowly without shaking. Stopped and allowed to stand overnight. The next day, the contents of the flask were shaken vigorously and filtered through Whatman No.1 filter paper, discarding the initial

10-15 ml of the filtrate. Aliquots of the filtrate were used for the estimation. Into three stoppered centrifuge tubes (test, standard and blank) pipetted out 1.5 ml of extract, 1.5 ml of the standard (D, L-α-tocopherol 10 mg/L in absolute alcohol, 91 mg of α-tocopherol is equivalent to 100 mg of tocopherol acetate) and 1.5 ml of water respectively. To the test and blank added 1.5 ml of ethanol and to the standard, added 1.5 ml of water. Added 1.5 ml of xylene to all the tubes, stoppered, mixed well and centrifuged. Transferred 1.0 ml of xylene layer into another stoppered tube. Added 1.0 ml of 2, 2'-dipyridyl reagent (1.2 g/L n-propanol) to each tube, stoppered and mixed. Pipetted out 1.5 ml of the mixture into spectrophotometer cuvettes and read the extinction of test and standard against the blank at 460 nm. Then, in turn, beginning with the blank, added 0.33 ml of ferric chloride solution. Mixed well and after exactly 15 minutes read the test and standard against the blank at 520 nm.

Estimation of flavonoids

0.1 ml of methanolic extract of plant sample was added to 0.3 ml of distilled water. To this 0.03 ml of 5% sodium nitrate was added to the tubes and incubated for 5 min at 25°C. After incubation, 0.03 ml of 10% aluminium chloride was added and again incubated for 5 min. To this 1mM sodium hydroxide (0.2ml) was added and made up to 1.0 ml with distilled water. The absorbance readings at 510 nm were noted. The final absorbance of each sample was compared with a standard curve made from catechin. From the standard graph, the amount of flavonoids present in the sample was calculated.

Estimation of Reduced glutathione

One gram of the sample was homogenized in 5 per cent TCA to give a 20 per cent homogenate. The precipitated protein was centrifuged at 1000 rpm for 10 minutes. The homogenate was cooled on ice and 0.1 ml of the supernatant was taken for the estimation. The volume of the aliquot was made up to 1.0 ml with 0.2M sodium phosphate buffer (pH 8.0). 2 ml of freshly prepared DTNB solution (0.6 mM in 0.2 M phosphate buffer, pH 8.0) was added to the tubes and the intensity of the yellow colour formed was read at 412 nm in a spectrophotometer after 10 minutes. The standards (2-10 nmoles GSH in 1.0 ml of 5 per cent TCA) were also treated in a similar manner.

Statistical analysis

All experimental data were expressed as mean ± SD. The results were analyzed by two way analysis of variance (ANOVA). Significant difference was accepted at the p<0.05 level.

RESULTS AND DISCUSSION

Phytochemical Screening of *Cleome viscosa* and *Trichodesma indicum*

The aqueous extracts of the *Cleome viscosa* and *Trichodesma indicum* was analysed qualitatively for the presence of phytochemicals such as alkaloids,

polyphenols and flavonoids. The results are presented in Table 1.

It was found that all the phytochemicals analysed were present in both the fruit and root of *Cleome viscosa* and

Trichodesmaindicum. The leaves of *Cleome viscosa* and roots of *Trichodesmaindicum* did not show the presence of alkaloids. The leaves of *Trichodesmaindicum* did not found to contain the flavonoids.

Table 1: Screening of phytochemicals in *Cleome viscosa* and *Trichodesmaindicum*

Phytochemicals	<i>Cleome viscosa</i>			<i>Trichodesmaindicum</i>		
	Leaf	Fruit	Root	Leaf	Fruit	Root
Alkaloids						
Mayer's test	-	+	+	+	+	-
Dragondroff's test	-	+	+	+	+	-
Wagner's test	-	+	+	+	+	-
Polyphenols						
Ferric chloride test	+	+	+	+	+	+
Lead acetate test	+	+	+	+	+	+
Flavonoids						
Concentrated H ₂ SO ₄ test	+	+	+	-	+	+
NaOH test	+	+	+	-	+	+
Schinodo's test	+	+	+	-	+	+

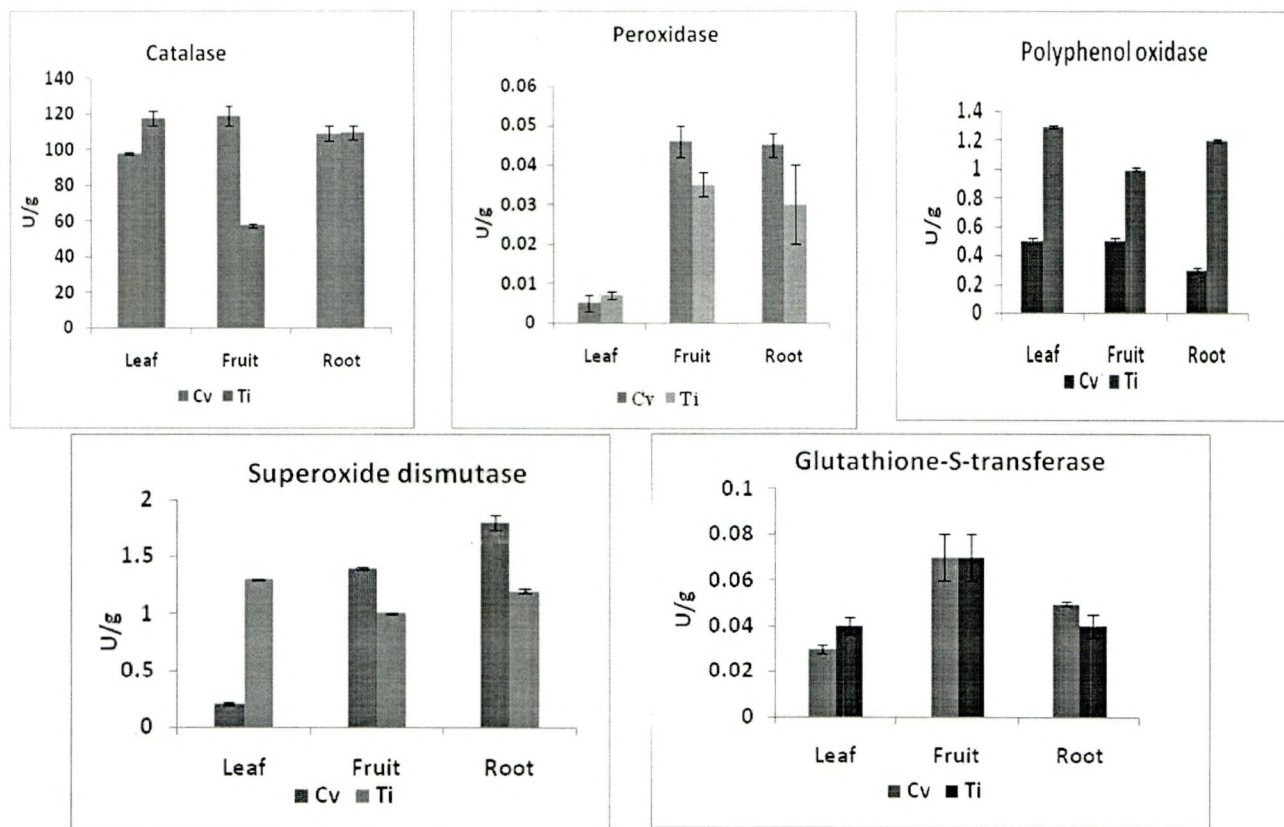


Figure 1: Activities of enzymic antioxidants in *Cleome viscosa* and *Trichodesmaindicum*

Antioxidant potential of *Cleome viscosa* and *Trichodesmaindicum*

Enzymic antioxidants

Catalase and peroxidase activity

Figure 1 presents the catalase and peroxidase activity of *Cleome viscosa* and *Trichodesmaindicum*. Catalase (CAT) a

tetrahedral protein constituted by four heme groups in the plants destroys hydrogen peroxide into oxygen and water in high concentration by catalyzing its two-electron dismutation.⁹

Figure 1 reveal that the *Cleome viscosa* and *Trichodesmaindicum* possess strong catalase activity than that of the peroxidase. Among the *Cleome viscosa* and

Trichodesma indicum, the fruits of *Cleome viscosa* was found to show significant ($p < 0.05$) catalase activity than the fruits of the *Trichodesma indicum*. The leaves of *Trichodesma indicum* shows significant ($p < 0.05$) catalase activity than that of leaves of *Cleome viscosa*. The catalase activity of *Cleome viscosa* was significantly higher ($p < 0.05$) in fruits (118 ± 5.6 U/g) followed by root (108 ± 4.4 U/g) and then leaves (97 ± 0.7) respectively. The catalase activity of *Trichodesma indicum* was significantly higher ($p < 0.05$) in leaves (117.5 ± 4.1 U/g) followed by root (109.5 ± 3.8) and then fruits (57.5 ± 0.9).

The catalase activity of *Coleus forskohlii* was significantly higher ($P < 0.05$) in tubers (5586.06 units/g dry tissue) followed by roots (2258.28 units/g dry tissue), leaves (1131.40 units/g dry tissue) and stem (1118.52 units/g dry tissue) respectively. Peroxidase activity was also found to be significantly higher ($P < 0.05$) in tubers (56.16 min/g dry tissue) than in the roots (42.33 min/g dry tissue), stem (34.31 min/g dry tissue) and leaves (23.25 min/g dry tissue).¹⁰

Polyphenol oxidase, superoxide dismutase and glutathione-S-transferase activity

Figure 1 indicate the polyphenol oxidase and superoxide dismutase activity of *Cleome viscosa* and *Trichodesma indicum*. It is evident from Figure 1 that the *Cleome viscosa* and leaf, fruit, root of *Trichodesma indicum* shows significantly moderate activity for the superoxide dismutase and polyphenol oxidase activity ($p < 0.05$).

The polyphenol oxidase activity in *Enicostemma littorale* was found to be in considerable quantity and was correlated to the degree of browning of fruits.¹¹ The results of enzymic antioxidant levels show a higher superoxide dismutase activity in the *Adhatodavasic*

(8.876 ± 0.696 units/mg protein) than *Sesbania grandiflora* (7.423 ± 0.846 units/mg protein).¹²

It was evident from Figure 1 that the glutathione-S-transferase activity of fruits of *Cleome viscosa* and *Trichodesma indicum* are significantly high (0.07 ± 0.01) than that of other parts of the plants.

Non enzymic antioxidants

Apart from enzymic antioxidants, a spectrum of non enzymic antioxidants is also important in cellular system in curtailing reactive oxygen species. The levels of the non enzymic antioxidants were assessed and the results are discussed as follows

Figure 2 reveals that the *Cleome viscosa* and *Trichodesma indicum* possess the significant ($p < 0.05$) level of ascorbic acid than the α -tocopherol. The figure shows that the leaves of both *Cleome viscosa* and *Trichodesma indicum* found to have maximum ascorbic acid content followed by fruits and roots. The minimum level of α -tocopherol is present in the root of *Trichodesma indicum*.

The present investigation also reveals that the α -tocopherol content of the leaves, fruits and roots of *Cleome viscosa* was found to be 1.1 ± 0.06 , 1.6 ± 0.04 and 0.9 ± 0.02 whereas in *Trichodesma indicum* the leaves contain 1.8 ± 0.05 , the fruits 1.2 ± 0.18 and roots 0.5 ± 0.36 respectively. Vitamin E content of two Serbian *Equisetum* species such as *E. fluviatile* and *E. palustre* were found to be 0.81 ± 0.01 and 0.51 ± 0.01 mg/g respectively.¹³

Figure 2 reveals that levels of *Cleome viscosa* and *Trichodesma indicum* shows significant ($p < 0.05$) levels of flavonoids and reduced glutathione. The flavonoids were found to be present in significantly lesser in the roots of *Cleome viscosa* and *Trichodesma indicum*. The leaf tissues of *Hibiscus* exhibited high flavonoid content (1.27 mg/g).¹⁴

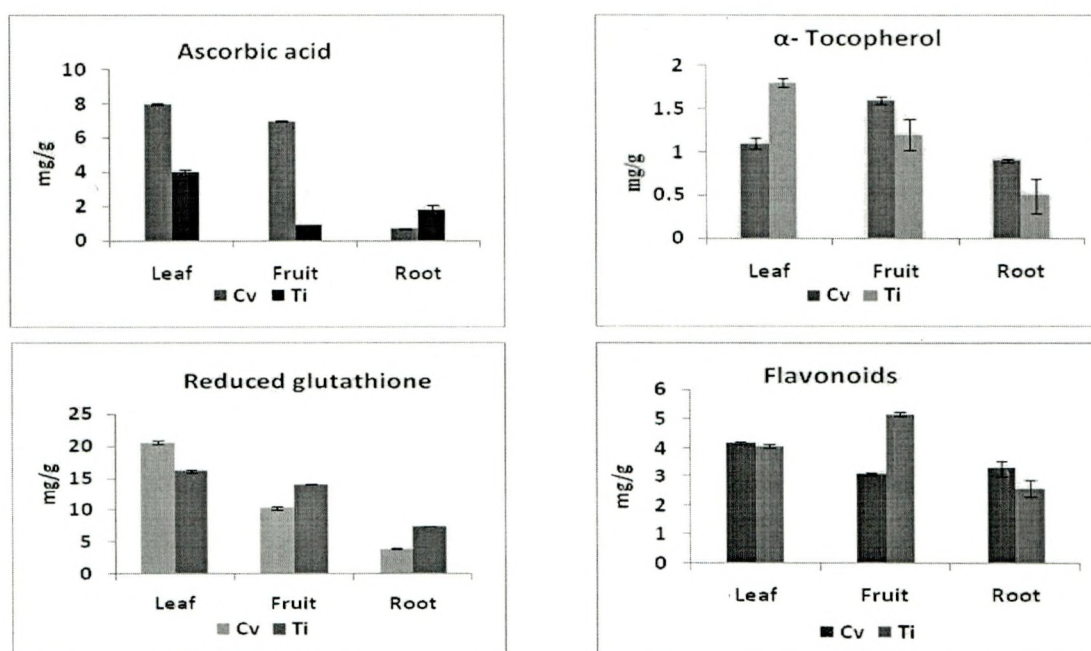


Figure 2: Levels of Non-enzymic antioxidants activity in *Cleome viscosa* and *Trichodesma indicum*



Plate I: *Cleome viscosa*



Plate I: *Trichodesma indicum*

CONCLUSION

It is evident from the above study that the leaf, fruit and root extracts of *Cleome viscosa* and *Trichodesma indicum* possess good antioxidant activity. Hence the plant can be further explored as a potent therapeutic agent for free radical related disorders.

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