

**Research Article****In vitro antioxidant activity of *Coccinia indica***

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**Abstract**

**Objective:** The antioxidant potential of different extracts was evaluated using DPPH free radical scavenging assay. **Material and methods:** Three different concentrations of extracts were prepared i.e. 1000µg/ml, 500µg/ml and 250µg/ml. Ascorbic acid was used as the standard. All the extracts demonstrated radical scavenging activity in a concentration dependent manner. **Results and conclusion:** The methanol extract of cultivated short variety exhibited the highest radical scavenging activity with 92.44% of inhibition. The percentage of inhibition increased with the increasing polarity of the solvents used for extraction. The fruits of three varieties showed highest percentage of inhibition followed by leaf, root and stem. The cultivated short variety extracts inhibited the free radicals more effectively when compared to wild and hybrid extracts, except in the case of leaves where wild extracts were more effective.

**Keywords:** *Coccinia indica*, antioxidant activity, solvents, DPPH, free radical

**Introduction**

Recently there has been an upsurge of interest in antioxidants obtained from plants because of the serious concern of carcinogenic potential of synthetic antioxidants. Reactive oxygen species (ROS), such as super oxide anions and hydroxyl radicals, can induce carcinogenesis and other pathological diseases by acting as initiating or promoting agents (Lee et al., 2001; Lakshmi et al., 2003). Therefore, the inactivation or elimination of ROS may be critical in preventing these diseases.

The antioxidant effect of an ethanolic extract of *Coccinia indica* leaves was studied in streptozocin diabetic rats by Venkateswaran and Pari (2003). Oral administration of *Coccinia indica* leaf extract (CLEt) (200 mg/kg body weight) for 45 days resulted in a significant reduction in thiobarbituric acid reactive substances and hydroperoxides. The effect of CLEt at 200 mg/kg body weight was more effective than glibenclamide. They showed that CLEt possesses antioxidant activity, which may be attributed to its protective action on lipid peroxidation and to the enhancing effect on cellular antioxidant defense contributing to the protection against oxidative damage in streptozotocin diabetes. Similar reports were given by Poungrat et al. (2003).

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Savitree et al. (2004) evaluated the DPPH free radical scavenging activity of methanol extract of fruits of *Coccinia indica* and also determined the total phenolic content. The results revealed that higher concentration of phenolic content is associated with higher radical scavenging activity. The antioxidant activity of *Coccinia indica* was investigated using β carotene bleaching method by Anchana et al. (2005). The content of plant chemicals such as vitamin C, vitamin E, carotenoids, tannins and total phenolics were also determined. Correlation between the chemical content and antioxidant index was observed and they suggested that chemicals such as vitamin C, vitamin E, carotenoids and phenolic compounds are the contributors to the antioxidant activity in the plants.

Umamaheshwari and Chatterjee (2008a) investigated the antioxidant activities of the various fractions of the hydromethanolic extract of the leaves of *Coccinia grandis* L. Voigt. (Cucurbitaceae) by using nine *in vitro* assays and were compared to standard antioxidants such as ascorbic acid, α-tocopherol, curcumin and butylated hydroxyl toluene (BHT). All the fractions showed effective H-donor activity, reducing power, free radical scavenging activity, metal chelating ability and inhibition of β-carotene bleaching. The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic and flavonoid compounds present in the fractions. They also suggested that the leaves of *Coccinia indica* are a potential source of natural antioxidants.

Investigations were carried out on several fractions of *Coccinia indica* L. Voigt on lipid peroxidation and antioxidant enzymes in oxonate-induced hyperuricaemic mice by Umamaheshwari and Chatterjee (2008c). Pretreatment with the petroleum ether, chloroform and ethyl acetate fractions of *Coccinia indica* prevented a rise in MDA and LH significantly ( $P < 0.01$ ) and enhanced the total protein and antioxidant enzymes. Among the fractions tested, the chloroform fraction exhibited highest activity followed by the petroleum ether and ethyl acetate fractions. They also suggested that the use of leaves of *C. grandis* for the treatment of gout and related inflammatory disorders could be attributed to its antioxidant activity.

Recently there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones. Natural antioxidant substances are presumed to be safe since they occur in plant foods, and are seen as more desirable than their synthetic counterparts. Data from both scientific reports and laboratory studies show that plants contain a large variety of substances called “plant chemicals” or “phytochemicals” that possess antioxidant activity. Natural antioxidants occur in all higher plants, and in all parts of the plant (wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen and seeds).

Oral administration of *Coccinia indica* leaf extract (CLEt) (200 mg /kg body weight) for 45 days resulted in a significant reduction in thiobarbituric acid reactive substances and hydroperoxides, and also showed that CLEt was more effective than glibenclamide (Venkateshwaran and Pari, 2003). Anchana et al. (2005) investigated the antioxidant activity of edible plants of Thailand including *Coccinia indica* and also determined the content of plant chemicals, such as vitamin C, vitamin E, carotenoids, tannin and total phenolics. They found that there was a correlation between the chemical content and the antioxidant index, and these antioxidant compounds are the contributors to antioxidant activity. Chanwitheesuk et al. (2005) reported the antioxidant activity of *Coccinia indica* and other plants. The antihyperglycemic and antioxidative potential of *Coccinia indica* was assessed in alloxan induced diabetic rats by Paliwal and Khemani (2006) and found that *Coccinia indica* not only reduced the oxidative stress but also strengthened the antioxidative potential. Eight compounds isolated from the fruits of *Luffacyl indrica* (L.) Roem were tested for their antioxidant activity using DPPH assay by Qizhen et al. (2006). Shahin et al. (2007) reviewed the antioxidant properties of 24 plants including *Coccinia indica*. Shu and Teik (2008) investigated the antioxidant activity of aqueous and ethanol extract of fruits of *Momordica charantia* using DPPH assay as well as  $FeCl_2$ -ascorbic acid induced lipid peroxidation assay in rat liver homogenates *in vitro*. Effat et al. (2008) studied the antioxidant activity and radical scavenging activity of methanolic extracts of selected Iranian plants including *Citrullus colocynthis* used in folk medicine against linoleic acid

peroxidation and DPPH radical. Marianna et al. (2009) screened extracts of four commercially available pumpkin seeds using DPPH free radical scavenging assay and for their inhibitory activity against lipid peroxidation catalyzed by soybean lipoxygenase and found that the extracts demonstrated radical scavenging activity depending on their phenolic content.

## Material and Methods

### Antioxidant activity

#### Preparation of sample

Three dilutions of different solvent extracts of fruits, leaves, stem and roots were prepared in methanol (250 µg/ml, 500 µg/ml and 1000 µg/ml).

#### DPPH free radical scavenging assay

The antioxidant activity of extracts was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH according to the method described by Adam and Piotrowska (2006). 200 µl aliquots of extract at different concentrations was mixed with 1.8 ml of the methanolic DPPH solution (0.5 mM). The reaction mixture was allowed to stand at room temperature for 30 minutes and absorbance was measured at 517 nm using UV-VIS spectrophotometer. Control solution consisting of DPPH and methanol without the extract was used as blank. Ascorbic acid was used as standard. The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with the extract) using the formula.

$$\% \text{ Inhibition} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100$$

Where  $A_{\text{control}}$  is the absorbance of DPPH and methanol solution without extract and  $A_{\text{sample}}$  is the absorbance of extract.

#### Preparation of sample / test solution

Different solvent extracts of fruits, leaves, stem and roots were used to prepare the test solution at three different concentrations i.e. 50 mg/ml, 100 mg/ml and 200 mg/ml in DMSO.

#### *In vitro* Xanthine Oxidase Inhibitory activity (XOI)

The XOI activity was assayed spectrophotometrically under aerobic conditions (Umamaheshwari et al., 2007). The assay mixture consisted of 1 ml of test solution, 2.9 ml of phosphate buffer (pH 7.5) and 0.1 ml of enzyme solution (0.01 units /1ml in phosphate buffer, pH 7.5), which was prepared immediately before use. After preincubation at 25°C for 15 minutes, the reaction was initiated by the addition of 2 ml of substrate solution (150 mM xanthine in

the same buffer). The assay mixture was incubated at 25°C for 30 minutes. The reaction was then stopped by the addition of 1ml of 1N Hydrochloric acid, and the absorbance was measured at 290 nm using a UV-spectrophotometer. A blank was also prepared in the same way, but the enzyme solution added to the assay mixture after adding 1N Hydrochloric acid. The assay was done in triplicate. One unit of XO is defined as the amount of enzyme required to produce 1m mol of uric acid per min at 25°C. XO activity was expressed as the percentage inhibition of xanthine oxidase in the above assay system, calculated as:

$$\% \text{ Inhibition} = \frac{\{(A - B) - (C - D)\}}{A - B} \times 100$$

Where, A is the activity of the enzyme without the extract, B the control of A without test extract and enzyme, C and D are the activities of the test extract with and without xanthine oxidase showed greater percentage of DPPH free radical inhibition.

### Statistical analysis

Percent of inhibition was calculated from the mean values of data.

### Results and discussion

There is an increased evidence for the participation of free radicals in the etiology of various diseases like cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases, aging, etc. (Bandopadhyay et al., 1999). A free radical is defined as any atom or molecule possessing unpaired electrons. The primary oxygen derived free radicals are superoxide anion ( $O_2^{\cdot-}$ ), hydroxyl ( $OH^{\cdot}$ ), hydroperoxyl ( $OOH^{\cdot}$ ), peroxy ( $ROO^{\cdot}$ ) and alkoxy ( $RO^{\cdot}$ ) radicals and non free radicals are hydrogen peroxide ( $H_2O_2$ ), hypochlorous acid ( $HOCl$ ), ozone ( $O_3$ ) and singlet oxygen ( $^1O_2$ ). These reactive intermediates are collectively termed as reactive oxygen species (ROS). Similarly, reactive nitrogen species (RNS) are mainly nitric oxide ( $NO^{\cdot}$ ), peroxy nitrite ( $ONOO^{\cdot}$ ) and nitrogen dioxide ( $NO_2$ ). Free radicals and reactive oxygen species (ROS) are formed during normal oxidative metabolism and pathological conditions in addition to exogenous factors. Free radicals can cause a wide range of toxic oxidative reactions like initiation of the peroxidation of the membrane lipids leading to the accumulation of lipid peroxides, direct inhibition of mitochondrial respiratory chain enzymes, fragmentation or random cross linking of molecules like DNA, enzymes and proteins which ultimately leads to cell death (Halliwell and Gutteridge, 1999). ROS can be formed in living organisms by both endogenous and exogenous sources. Endogenous sources of free radicals include normal aerobic respiration, peroxisomes and stimulation of polymorphonuclear leucocytes and macrophages. The exogenous sources include ionizing radiation, tobacco smoke, environmental pollutants, pesticides, organic

solvents, chemicals, toxins, deep fried and spicy foods as well as physical stress (Irshad and Chaudhuri, 2002).

Oxidation is essential for many living organisms for the production of energy to fuel biological processes. Cells in the human body use oxygen to break down carbohydrates, proteins and fats that give them energy. Metabolically active cells produce by-products called free radicals. These are atoms or groups of atoms that have at least one unpaired electron, which makes them highly reactive. They promote beneficial oxidation that produces energy and kill bacterial invaders. If free radicals are at reasonable levels, the human body produces enzymes to combat them. When metabolic free radicals are added to exogenous factors, they cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins, which cause oxidative stress, a condition in which the body's natural defenses are over run. Oxidative stress has been implicated in the pathology of more than hundred disorders in humans including atherosclerosis, arthritis, ischemia, reperfusion injury of many tissues, central nervous system injury, gastritis, cancer, AIDS, multiple sclerosis, creutzfeldt-jacob disease, meningoencephalitis, cardiovascular disease (CVD) and cardiac failure, rheumatoid arthritis and other degenerative processes associated with aging (Pourmorad et al., 2006).

Antioxidants are agents which scavenge the free radicals and prevent the damage caused by them. They can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA (Fang et al., 2002). Antioxidants act as radical scavengers, hydrogen donors, peroxide decomposers, electron donors, enzyme inhibitors, singlet oxygen quenchers, synergist and metal chelating agents. Antioxidants can be classified into two major classes i.e., enzymatic and non-enzymatic. The enzymatic antioxidants are produced endogenously and include superoxide dismutase, catalase, and glutathione peroxidase. These convert hydrogen peroxide and hydroperoxide to non-radical forms and function as natural antioxidants in human body. The non-enzymatic antioxidants include tocopherols, carotenoids, ascorbic acid, flavonoids and tannins which are obtained from natural plant sources (Lee et al., 2004). A wide range of antioxidants from both natural and synthetic origin have been proposed for use in the treatment of various human diseases (Cuzzocrea et al., 2001). There are some synthetic antioxidant compounds such as butylatedhydroxytoluene, butylatedhydroxyanisole and tertiary butylhydroquinone which are commonly used in processed foods. However, it has been suggested that these compounds have shown toxic

effects like liver damage and mutagenesis (Grice, 1986; Wilchi, 1988). Flavonoids and other phenolic compounds of plant origin have been reported as scavengers of free radicals (Formica and Regelson, 1995; Rice-Evans et al., 1997). Hence, now-a-days search for natural antioxidant source is gaining much importance.

Typical compounds that exhibit antioxidant activity include vitamins, carotenoids, and phenolic compounds. Therefore, recommendations have been made to increase the daily intake of fruit and vegetables, which are rich in these nutrients that lower the risk of chronic health problems associated with the diseases mentioned above (Slattery et al., 2000).

High dietary intake of fruits and vegetables lowers the risk of most types of cancer (Ames, 1983). It is important to note that most of the investigations regarding inhibitory effect of food component on the oxidative damages of biomolecules such as DNA, lipids, and proteins have been devoted to the foods of plant origin. Several studies have reported the antimutagenic, antioxidant and anticarcinogenic activities of extracts from fruits, vegetables and cereals (Bala and Grover, 1989; Vayalil, 2002). Moreover, natural antioxidants from plant extracts have attracted the attention due to consumer concern about the safety of the synthetic antioxidants in food. Recently, extracts of fruits, vegetables, herbs, cereals, sprouts and seeds showed antioxidant activity in different model systems (Kahkohen et al., 2001).

The antioxidant compounds of higher plants have been demonstrated, through *in vitro* experiments, to protect against oxidation damage by inhibiting or quenching free radicals and reactive oxygen species. The role of these compounds as potential antioxidants can be inferred by their similarity to synthetic antioxidants, of related structures (Larson, 1988). Vitamin C has been proposed, for a long time, as a chain-breaking scavenger for peroxy radicals and also to act as a synergist with vitamin E, since vitamin C can donate a hydrogen atom to the vitamin E- derived phenolate radical, thus regenerating its activity. Vitamin E is one of the best quenchers for singlet oxygen, and can act as a chain-breaking antioxidant. Furthermore, singlet oxygen is very powerfully quenched by carotenoids, especially  $\beta$ -carotene. In the case of phenolic compounds, the ability of the phenolics to act as antioxidants depends on the redox properties of their phenolic hydroxyl groups, that allow them to act as reducing agents, hydrogen-donating antioxidants and oxygen quenchers (Rice-Evans and Miller, 1996).

The strong association between fruit and vegetable intake and degenerative disease prevention has been explained by the aspect of antioxidant phyto nutrients. In recent years particular attention has been given to a specific class of antioxidant phytochemicals, the polyphenols. These polyphenols comprise basically of

phenolic acids including benzoate and hydroxycinnamate derivatives, and flavonoids. Polyphenolic substances are naturally present in vegetables and fruits. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Frankel, 1995). Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski et al., 1987).

Nutraceuticals are supposed to hold the key to a healthy society in the coming future. Antioxidants derived from fruits, vegetables, spices and cereals are very effective and have reduced interference with the body's ability to use free radicals constructively (Kahkonen et al., 1999; Wolfe et al., 2003). Natural antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid and carotenoids. The quest for natural antioxidants for dietary, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenge over the last two decades. Efforts to gain extensive knowledge regarding the power of antioxidants from plants and to tap their potential are therefore on the increase.

Numerous findings have been reported on the antioxidant properties of *Coccinia indica* by different researchers. Poungrat et al. (2003) screened freeradical scavenging activities of extracts from *Coccinia grandis* and *Centella*. The antioxidant effect of an ethanolic extract of leaves of *Coccinia indica* was studied in streptozotocin – diabetic rats by Venkateshwaran and Pari (2003). Oral administration of *Coccinia indica* leaf extract (CLEt) (200 mg/kg body weight) for 45 days resulted in a significant reduction in thiobarbituric acid reactive substances and hydroperoxides, and also showed that CLEt was more effective than glibenclamide. Anchana et al. (2005) investigated the antioxidant activity of edible plants of Thailand including *Coccinia indica* and also determined the content of plant chemicals, such as vitamin C, vitamin E, carotenoids, tannin and total phenolics. They found that there was a correlation between the chemical content and the antioxidant index and these antioxidant compounds are the contributors to antioxidant activity.

Shahin et al. (2007) reviewed the antioxidant properties of 24 plants including *Coccinia indica*. Umamaheshwari and Chatterjee (2008a) investigated the *in vitro* antioxidant activities of petroleum ether, chloroform and ethyl acetate fractions extracted from hydromethanolic extract of leaves of *C. grandis*, and found that all the extracts showed effective antioxidant activity. The chloroform extract was most effective followed by ethyl acetate and petroleum

ether extract. Umamaheshwari and Chatterjee (2008c) investigated the effect of several fractions of *Coccinia grandis* L. Voigt on lipid peroxidation and antioxidant enzymes in oxonate-induced hyperuricaemic mice. Pretreatment with petroleum ether, chloroform and ethyl acetate fractions of *C. grandis* prevented a rise in MDA and LH significantly ( $P < 0.01$ ) and enhanced the total protein content and antioxidant enzymes. Among the fractions tested, the chloroform fraction exhibited highest activity followed by the petroleum ether and ethyl acetate fractions. It was suggested that the use of leaves of *C. grandis* for the treatment of gout and related inflammatory disorders could be attributed to its antioxidant property.

#### Antioxidant activity of fruit

Significant radical scavenging activity was evident at all the tested concentrations (Table 1). The cultivated variety methanol extract exhibited the highest radical scavenging activity with 92.44% of inhibition followed by hybrid (90.36%) and wild variety of extract (83.39%). The scavenging activity increased with increasing concentration. The ethyl acetate and acetone extracts also showed significant activity ranging from 65.92 – 87.50% followed by considerable activity of chloroform and petroleum ether extract with percentage of inhibition ranging from 46.96 – 69.17%. Aqueous extract inhibited free radical to a lesser extent compared to other extracts.

#### Antioxidant activity of leaf

All the fractions demonstrated radical scavenging activity in a concentration dependent manner (Table 2). Among the wild and cultivated variety, wild leaf extracts showed strong activity than cultivated variety extracts. The highest DPPH radical

scavenging activity was detected in methanol extracts (77.45–89.12%) with maximum percentage of inhibition, followed by ethyl acetate, chloroform, acetone, petroleum ether and aqueous extracts (59.74 – 77.33%, 51.64 – 69.41%, 38.17 – 57.18%, 27.33 – 44.51%, 20.14 – 31.69%). The scavenging activity of methanol and ethyl acetate extract was comparable with that of ascorbic acid at different concentrations (70.54–79.15%).

#### Antioxidant activity of stems

The methanol extract of cultivated stem showed strong inhibition of DPPH free radical when compared to all extracts (Table 3). The scavenging ability increased with the increasing polarity of the solvent. The anti-oxidant activity increased in the order of methanol > ethyl acetate > chloroform > acetone > petroleum ether > aqueous extracts. Percentage of inhibition was maximum form ethanol extracts i.e. 57.95 – 78.13% and comparable to that of ascorbic acid (70.43–79.27%). Other extracts also showed good antioxidant activity but at higher concentrations. The antioxidant activity of chloroform and acetone extracts was almost similar i.e. 47.31 – 59.47% and 31.85– 49.14% at 500 µg/ml and 1000 µg/ml.

#### Antioxidant activity of roots

All the fractions of wild and cultivated variety demonstrated H-donor Activity (Table 4). The methanol extract of cultivated variety exhibited the highest level of antioxidant activity (49.17 – 58.79%) when compared to wild variety methanol extract (41.26 – 51.36%). Ethyl acetate, acetone and chloroform extracts also showed significant scavenging

**Table 1.** Percentage of radical scavenging activity of fruits of *Coccinia indica*

| Concentration<br>µg/ml) | Petroleum ether |       |       | Chloroform |       |       | Ethyl acetate |       |       | Acetone |       |       | Methanol |       |       | Aqueous |       |       | Ascorbic<br>acid |
|-------------------------|-----------------|-------|-------|------------|-------|-------|---------------|-------|-------|---------|-------|-------|----------|-------|-------|---------|-------|-------|------------------|
|                         | C               | H     | W     | C          | H     | W     | C             | H     | W     | C       | H     | W     | C        | H     | W     | C       | H     | W     |                  |
| 1000                    | 69.17           | 63.4  | 62.96 | 68.5       | 64.19 | 62.82 | 87.5          | 83.19 | 81.42 | 77.39   | 74.21 | 70.48 | 92.44    | 90.36 | 83.39 | 44.21   | 43.17 | 38.22 | 79.11            |
| 500                     | 60.79           | 57.86 | 56.65 | 59.31      | 55.19 | 51.48 | 79.45         | 75.31 | 72.28 | 74.62   | 79.41 | 74.51 | 89.14    | 89.26 | 80.72 | 41.15   | 38.89 | 32.97 | 77.23            |
| 250                     | 54.88           | 56.72 | 49.17 | 51.49      | 47.32 | 46.96 | 74.31         | 69.12 | 67.46 | 70.32   | 71.64 | 65.92 | 85.47    | 84.36 | 75.41 | 37.91   | 36.12 | 28.33 | 70.74            |

C: Cultivated; W: Wild

**Table 2.** Percentage of radical scavenging activity of leaf of *Coccinia indica*

| Concentration<br>(µg/ml) | Petroleum ether |       | Chloroform |       | Ethyl acetate |       | Acetone |       | Methanol |       | Aqueous |       | Ascorbic acid |
|--------------------------|-----------------|-------|------------|-------|---------------|-------|---------|-------|----------|-------|---------|-------|---------------|
|                          | C               | W     | C          | W     | C             | W     | C       | W     | C        | W     | C       | W     |               |
| 1000                     | 41.38           | 44.51 | 62.32      | 69.41 | 70.12         | 77.33 | 44.9    | 57.18 | 86.99    | 89.12 | 24.37   | 31.69 | 79.15         |
| 500                      | 32.19           | 41.84 | 57.14      | 64.72 | 61.56         | 72.39 | 41.23   | 52.43 | 82.34    | 82.49 | 21.45   | 28.19 | 78.46         |
| 250                      | 27.33           | 32.97 | 51.64      | 62.85 | 59.74         | 67.91 | 38.17   | 46.75 | 77.45    | 79.53 | 20.14   | 22.91 | 70.54         |

C: Cultivated; W: Wild

**Table 3.** Percentage of radical scavenging activity of stem of *Coccinia indica*

| Concentration<br>(µg/ml) | Petroleum ether |       | Chloroform |       | Ethyl acetate |       | Acetone |       | Methanol |       | Aqueous |       | Ascorbic acid |
|--------------------------|-----------------|-------|------------|-------|---------------|-------|---------|-------|----------|-------|---------|-------|---------------|
|                          | C               | W     | C          | W     | C             | W     | C       | W     | C        | W     | C       | W     |               |
| 1000                     | 36.14           | 31.59 | 59.47      | 51.28 | 67.16         | 63.28 | 49.14   | 40.95 | 78.13    | 72.44 | 24.19   | 20.31 | 79.27         |
| 500                      | 29.66           | 25.51 | 52.16      | 47.31 | 61.31         | 57.47 | 41.46   | 31.85 | 72.19    | 64.37 | 22.87   | 17.34 | 75.17         |
| 250                      | 23.98           | 19.27 | 47.28      | 41.22 | 58.95         | 50.67 | 33.15   | 27.26 | 69.86    | 57.95 | 19.83   | 14.17 | 70.43         |

C: Cultivated; W: Wild

**Table 4.** Percentage of radical scavenging activity of root of *Coccinia indica*

| Concentration<br>(µg/ml) | Petroleum ether |       | Chloroform |       | Ethyl acetate |       | Acetone |       | Methanol |       | Aqueous |       | Ascorbic acid |
|--------------------------|-----------------|-------|------------|-------|---------------|-------|---------|-------|----------|-------|---------|-------|---------------|
|                          | C               | W     | C          | W     | C             | W     | C       | W     | C        | W     | C       | W     |               |
| 1000                     | 27.45           | 21.04 | 34.52      | 28.75 | 49.09         | 41.11 | 43.89   | 38.23 | 58.79    | 51.36 | 23.14   | 20.11 | 80.18         |
| 500                      | 22.14           | 19.56 | 31.48      | 22.51 | 47.85         | 41.51 | 40.26   | 33.64 | 52.42    | 48.81 | 19.23   | 12.47 | 72.11         |
| 250                      | 20.79           | 19.53 | 26.17      | 23.19 | 43.89         | 41.32 | 37.93   | 31.46 | 49.17    | 41.26 | 16.27   | 14.59 | 68.21         |

C: Cultivated; W: Wild

activity and percentage of inhibition of cultivated variety of these extracts at each concentration was higher than that of wild variety at that same concentration. Petroleum ether and aqueous extracts of both the varieties did not show considerable activity and were less than the ascorbic acid (68.21-80.18%).

Typical compounds that possess antioxidant activity have been characterized as vitamin C, carotenoids, phenolic compounds, flavonoids etc. It was reasonable to investigate their total level in different parts of cultivated and wild varieties. A correlation between content of phytochemicals and antioxidant index was found. Fruit extracts showed strongest scavenging activity and also contained higher phenolic content and other antioxidant compounds followed by leaf, root and stem of *Coccinia indica*.

This study documents for the first time the free radical scavenging ability of cultivated, hybrid and wild variety organic extracts and aqueous extract. There are no reports of comparison of wild and cultivated variety. To our knowledge there are no reports on antioxidant activity of extracts of fruit, stem and root of *Coccinia indica*. Though there are reports on petroleum ether, chloroform, ethyl acetate, ethanol, hydromethanolic and methanolic extracts of leaf of *Coccinia indica*, no work was done on acetone and aqueous extracts. Our investigations reported the antioxidant activity of these unexplored extracts for the first time.

### Conclusion

In the present investigation, the fruit, leaf, stem and root of cultivated (short and hybrid) and wild varieties of *Coccinia indica* were extracted successively with petroleum ether, chloroform, ethyl acetate, acetone, methanol and water using a Soxhlet apparatus for 8 hours each. The relative ability of these extracts to act as antioxidants was investigated through *in vitro*

radical scavenging activity at three different concentrations (i.e. 250 µg/ml, 500 µg/ml and 1000 µg/ml) using  $\alpha, \alpha$ -diphenyl  $\beta$ -picrylhydrazyl (DPPH) method. The present investigation revealed that the methanolic extract of fruit showed highest level of radical scavenging activity when compared to other extracts. As the various fractions of *C. grandis* exhibited different radical scavenging activities, there may be different percentages of phytochemical constituents present in the fractions. The results also indicate that selective extraction from natural sources, by an appropriate solvent is important for obtaining fractions with high antioxidant activity.

### Conflicts of interest

The authors declare there is no conflict of interests.

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