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In vitro Antioxidant profile of *Wedelia calandulaceae* leaves

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Abstract: Background: *Wedelia calandulaceae* Less. non-Rich. (Family: Compositae) is a traditional Ayurvedic herb. The plant has been used traditionally as a medicine as well as a functional food in several traditional medicinal recipes. The present study is to evaluate the *in vitro* antioxidant profile of methanolic extract of the leaves of this plant. **Materials and Method:** The *in vitro* antioxidant assays performed includes total antioxidant activity, reducing power assay, DPPH radical scavenging activity, superoxide anion radical scavenging activity, nitric oxide scavenging activity and hydrogen peroxide scavenging activity. **Results:** The total phenolic content was also estimated. Hydrogen peroxide scavenging activity of the extract was found to be the most potent when compared to the standard antioxidant. The IC_{50} (Half maximal inhibitory concentration) values of the extract were found to be 110.881, 175.292, 207.615 and 139.824 $\mu g\ mL^{-1}$ for DPPH radical scavenging activity, superoxide anion radical scavenging, nitric oxide scavenging activity and hydrogen peroxide scavenging activity, respectively. The various antioxidant activities were compared with standard antioxidants such as Butylated Hydroxyanisole, α-Tocopherol and ascorbic acid. **Conclusion:** The present study confirmed an antioxidant profile of this plant and is strongly in accordance with its use in Indian systems of medicine and also as a functional food.

Key words: Free radical scavenging, DPPH radical scavenging, total phenolic content, nitric oxide scavenging, superoxide anion

INTRODUCTION

Free radicals have been claimed to play a key role in understanding pathophysiology of different non-communicable diseases such as cardiovascular diseases, cancer, diabetes, neurodegenerative diseases etc. (Gerber *et al*., 1996; Kris-Etherton *et al*., 2002; Serafini *et al*., 2002). Free radicals contain unpaired electrons and can be generated during normal body metabolic function and also can be acquired from the environment.. The oxygen radicals, such as superoxide radical (O_2^{-}), Hydroxyl Radical ($^{\bullet}OH$) and non free radical species, such as Hydrogen Peroxide (H_2O_2) and singlet Oxygen ($^{\bullet}O_2$), are generated in many redox processes in the human physiological system (Gulcin *et al*., 2002). Superoxide dismutase, catalase and glutathione peroxidise etc are the enzymes which trap and destroy these free radicals. Vitamin A, vitamin C and vitamin E deficiency together with overproduction of free radicals and a reduced level of above mentioned enzymes, is considered as the main culprit for producing oxidative stress (Ellnain-Wojtaszek *et al*., 2003).

Free radicals mainly act by attacking the unsaturated fatty acids in the biomembranes which causes membrane lipid peroxidation, decrease in membrane fluidity and reduction of enzyme and receptor activity and damage to membrane protein which finally triggers the cell inactivation and death (Dhully *et al*., 1993). Oxidative stress by oxygen free radicals also cause serious damage to different biomolecules such as carbohydrates, proteins, lipids and DNA which lead to acceleration of aging, cancer, cardiovascular diseases, neurodegenerative diseases and inflammation (Fig. 1). Therefore, antioxidants can be used to reverse the harmful and pathological action of free radicals. There are two types of antioxidants-natural and synthetic antioxidants. Due to carcinogenic probability, synthetic antioxidants are not preferred type of antioxidants (Ito and Hirose, 1989). Current research in the field of free radical biology emphasizes the use of antioxidants from natural origin

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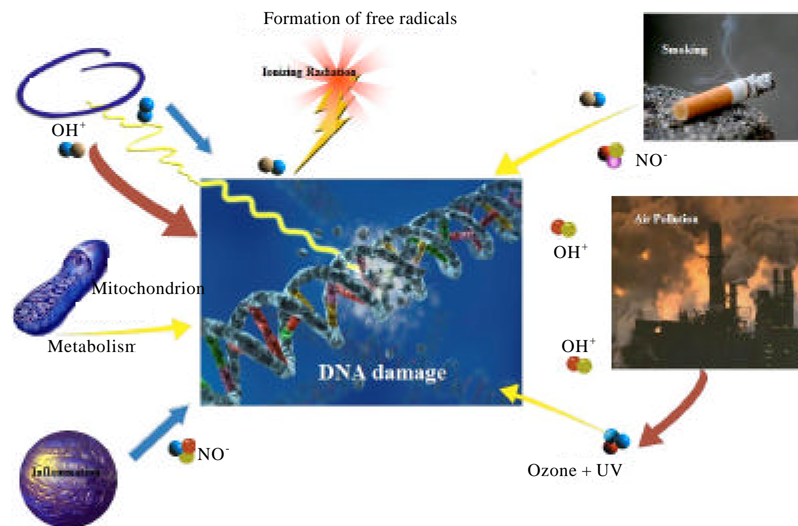

Fig. 1: A overview of different sources of free radicals and damage caused by it

therapeutically and also nutraceutically. As a result more and more antioxidants of natural origin are investigated.

Wedelia calandulaceae Less. non-rich. (synonym: *Wedelia chinensis* Merrill) (Family: Compositae) is a traditional Ayurvedic herb and also known as pitabhringa-raaja in Hindi, pitabhringi in Sanskrit, gargari in Kannada and manjal karisaalai in Tamil (Chopra *et al*., 1956; Khare, 2007). It is a perennial herb with bright yellow flowers and light camphor like odour occurring widely throughout India. The plant is also widely found in humid and coastal areas including Assam, Arunachal Pradesh, Himachal Pradesh, Uttar Pradesh and Tamilnadu (Chopra *et al*., 1956; Khare, 2007). The plant has been used traditionally as cholagogue, deobstruent, in uterine haemorrhage, in menorrhagia, as tonic for hepatic enlargement and in abdominal swellings. The juices of leaves are used as dyeing agent for hair, as hair growth promoter and in alopecia. Traditionally the leaves are also considered tonic, alternative and useful in cough, cephalalgia and several skin diseases. The plant is also used for viral hepatitis (Khare, 2007; Kirtikar and Basu, 2001; Prajapathi *et al*., 2004). According to the previous phytochemical works, the leaves of *Wedelia calandulaceae* contain wedelolactone and demethyl wedelolactone (Govindachari *et al*., 1956), isoflavonoids, bisdesmosidic oleanolic acid saponins and wedelolactones (Govindachari and Premila, 1985; Khare, 2007) and norwedelolactone (Bhargava *et al*., 1970). Norwedelic acid (Govindachari and Premila, 1985) and Kauren diterpenes (Haider *et al*., 2003) were also isolated from the leaves of *Wedelia calandulaceae*. 5-lipoxygenase and caspase inhibitory activities by wedelolactones from the plant were reported (Kobori *et al*., 2004; Wagner *et al*., 1986). Other activities reported by previous researchers include hepatoprotective activity (Sharma *et al*., 1989), wound healing activity (Hegde *et al*., 2006), sedative activity (Prakash *et al*., 2008) and post-menopausal anti-osteoporotic effect (Annie *et al*., 2006) of *Wedelia calandulaceae* in different animal models. The plant was found to possess antitumor activity against Ehrlich Ascites Carcinoma in mice (Gupta *et al*., 2007). A recent study also reported a cancer chemopreventive role of methanol extract of this plant against chemical carcinogenesis in Swiss Albino mice (Haldar *et al*., 2011). As far as our literature survey could ascertain; *in vitro* antioxidant activities of the plant extract have not been reported previously. Hence, the present study aims to investigate the antioxidant profile of *Wedelia calandulaceae* leaves in different *in vitro* models in order to aid the understanding of the usefulness of this plant as an alternative medicine.

MATERIALS AND METHODS

Plant material: Leaves of *Wedelia calendulacea* were collected from Tanda, Mandi district of Himachal Pradesh (India) and authenticated by Dr. S.K. Sharma, Botanist, Research Institute in Indian System of Medicine (ISM), Joginder Nagar, Mandi, Himachal Pradesh. A voucher specimen herbarium (MSK/ACP/09/2010) was deposited

in the Pharmacognosy department, Abhilashi College of Pharmacy, Mandi, Himachal Pradesh for future reference.

Drugs and chemicals: Chemicals, such as Folin-Ciocalteau reagent, Trichloroacetic Acid (TCA), ethanol, methanol, ammonium thiocyanate, Dimethylsulphoxide (DMSO), Gallic acid, Tween 20, α-Tocopherol, Butylated Hydroxyanisole (BHA) were purchased from E. Merck (India) Limited. Linoleic acids, Sulphanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride, ascorbic acid, riboflavin, NBT (Nitroblue tetrazolium), ferrozine, DPPH were procured from Sigma, USA. Petroleum ether, Tween 80 and all other chemicals and solvents used were of analytical grade available commercially (SRL Mumbai, Himedia, E.Merck India).

Preparation of extracts: The leaves of *Wedelia calendulacea* were air dried and then powdered. The powdered plant leaves were extracted with methanol using Soxhlet apparatus and concentrated *in-vacuo*. Approximately, 2.2 gm of dried methanolic extract of *Wedelia calendulacea* leaves (MEWC) was obtained from 25 g of dried leaf material (Yield, 8.8%).

Preliminary phytochemical screening: Identification of the chemical constituents were carried out on the powdered drug and on the methanolic extract using chemical methods according to the methodology proposed elsewhere (Harborne, 1973).

Determination of total phenolic content: Total soluble phenolics in the leaf extract of the plant were determined with Folin-Ciocalteau reagent according to the method using gallic acid as a standard phenolic compound (Slinkard and Singleton, 1977). About 1.0 mL of extract solution containing 10 mg extract in a volumetric flask was diluted with 46 mL of distilled water. About 1.0 mL of Folin-Ciocalteau reagent was added and mixed thoroughly. Three minutes later 3.0 mL of 2% sodium carbonate was added and the mixture was allowed to stand for 3 h with intermittent shaking. The absorbance of the mixture was measured at 760 nm in a spectrophotometer (UV-1601 Shimadzu, Japan). The concentration of total phenols was expressed as mg g^{-1} of extract. The concentration of total phenolic compounds in the extract was determined as gram of Gallic Acid Equivalent (GAE) using an equation obtained from the standard gallic acid graph:

$$Y = 0.002\,x + 0.070,\ R^2 = 0.9550$$

where, Y was the absorbance and x was the concentration.

Total antioxidant activity: The total antioxidant activity of the extract was determined according to the thiocyanate method (Mitsuda *et al*., 1996). Ten milligrams of extract was dissolved in 10 mL water. Different concentration of extract (50-250 μg mL^{-1}) or standard samples in 2.5 mL of potassium phosphate buffer (0.04 M, pH 7.0) was added to linoleic acid emulsion (2.5 mL) in potassium phosphate buffer (0.04 M, pH 7.0). Five millilitres linoleic acid emulsion consists of 17.5 g Tween-20, 15.5 μL linoleic acid and 0.04 M potassium phosphate buffer (pH 7.0). On the other hand, 5.0 mL control consists of 2.5 mL linoleic acid emulsion and 2.5 mL potassium phosphate buffer (0.04 M, pH 7.0). The mixed solution was incubated at 37°C in a glass flask and in the dark. After the mixture was stirred for 3 min, the peroxide value was determined by reading the absorbance at 500 nm in a spectrophotometer (UV-1601 Shimadzu, Japan), after reaction with $FeCl_2$ and thiocyanate at intervals during incubation. During the linoleic acid oxidation, peroxides formed. These compounds oxidize Fe^{2+} to Fe^{3+}. The latter Fe^{3+} ions form complex with SCN-which had maximum absorbance at 500 nm. Therefore, high absorbance indicates high linoleic acid oxidation. The solutions without extract or standards were used as blank samples. All data about total antioxidant activity are the average of triplicate analyses. The inhibition of lipid peroxidation in percentage was calculated by following equation:

$$\text{Inhibition (\%)} = (A_0\text{-}A_t/A_0) \times 100$$

where, A_0 was the absorbance of the control reaction and A_t was the absorbance in the presence of the sample. All the tests were performed in triplicate and graph was plotted with the Mean±SD values. BHA and α-Tocopherol were used as standard antioxidant compounds.

Reducing power: The reducing power of extract was determined according to the method described previously (Oyaizu, 1986). The different concentrations of extract (50-250 μg mL^{-1}) in 1 mL of distilled water was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm in a spectrophotometer (UV-1601 Shimadzu, Japan). Higher absorbance of the reaction mixture indicated greater reducing power. BHA and α-Tocopherol were used as standard antioxidant compounds.

Determination of DPPH (1-1-diphenyl-2-picryl hydrazyl) radical scavenging activity: The free radical scavenging activity of extract was measured by DPPH• using the method described previously (Shimada *et al*., 1992). A 0.1 mM solution of DPPH• in ethanol was prepared and 1 mL of this solution was added to 3 mL of extract solution in water at different concentrations (50-250 µg mL^{-1}). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer (UV-1601 Shimadzu, Japan). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH scavenging effect was calculated using the following equation:

$$DPPH^{\bullet} \text{ scavenging effect } (\%) = 100-[(A_0-A_t/A_0) \times 100]$$

where, A_0 was the absorbance of the control reaction and A_t was the absorbance in the presence of the standard sample or extract. All the tests were performed in triplicate and graph was plotted with the Mean±SD values. BHA was used as standard antioxidant compound.

Assay of superoxide radical ($O_2^{\bullet-}$) scavenging activity: The assay was based on the capacity of the methanolic extract to inhibit blue formazon formation. Superoxide radical were generated in riboflavin-light-NBT (Nitroblue tetrazolium) system (Beauchamp and Fridovich, 1971). The total volume of the reactant mixture was 3 mL. Each 3 mL of this reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 µg riboflavin and 12 mM EDTA and 0.1 mg NBT and 1 mL sample solution. Reaction was started by illuminating the reaction mixture with different concentrations of the methanolic plant extract (50-250 µg mL^{-1}) for 90 sec. Immediately after illumination, the absorbance was measured at 590 nm. The reaction assembly was enclosed in a aluminium foil lined box. Unilluminated identical tubes containing reaction mixture served as blank. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\text{Inhibition } (\%) = (A_0-A_t/A_0) \times 100$$

where, A_0 was the absorbance of the control (without extract) and A_t was the absorbance in the presence of the extract or standard. All the tests were performed in triplicate and graph was plotted with the Mean±SD values. Ascorbic acid was used as standard compound.

Assay of nitric oxide scavenging activity: The procedure is based on Greiss reaction (Kumaran and Karunakaran, 2006; Sreejayan and Rao, 1997). Sodium nitroprusside spontaneously generates nitric oxide at physiological pH in aqueous solution which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. Sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with different concentrations of methanolic extract of the plant dissolved in methanol and then incubated at room temperature for 150 min. In the same way, a reaction mixture was prepared without the methanolic extract but with equivalent amount of methanol was added. This served as control. After the incubation period, 0.5 mL of Greiss reagent (1% Sulphanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added to the mixture. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthyl ethylenediamine was measured at 546 nm. Standard solutions of ascorbic acid treated in the same way as tests with Greiss reagent served as positive control. The percentage of inhibition was calculated by using the following formula:

$$\text{Inhibition } (\%) = (A_0-A_t/A_0) \times 100$$

where, A_0 was the absorbance of the control (without extract) and A_t was the absorbance in the presence of the extract. All the tests were performed in triplicate and graph was plotted with the Mean±SD values.

Hydrogen peroxide scavenging activity: Hydrogen peroxide (H_2O_2) scavenging ability of the extract was measured using a method described previously (Ruch *et al*., 1989). A solution of hydrogen peroxide (2 mmol L^{-1}) was prepared in phosphate buffer (pH 7.4). Concentration of hydrogen peroxide was determined spectrophotometrically from absorption at 230 nm with molar absorbtivity 81 (mol L^{-1}) cm^{-1}. The plant extract (50-250 µg mL^{-1}) were added to H_2O_2 solutions (0.6 mL). Absorbance of H_2O_2 at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without H_2O_2. The percentage inhibition was calculated using the following formula:

$$\text{Inhibition } (\%) = (A_0-A_t/A_0) \times 100$$

where, A_0 was the absorbance of the control (without extract) and A_t was the absorbance in the presence of the extract or standard. All the tests were performed in triplicate and graph was plotted with the Mean±SD values. Ascorbic acid was used as standard compound.

RESULTS AND DISCUSSION

Preliminary phytochemical screening: The powdered leaves subjected to preliminary phytochemical screening using different chemical methods showed the presence of phytosterols, saponins, flavanoids, tannins, hydrolysable tannins, phenolic compounds and proteins and amino acids. The test for alkaloid showed negative result. Similar phytochemical screening of the methanolic extract of the powdered leaves of the plant indicated the presence of alkaloids, saponins, phytosterols, flavanoids, tannins, phenolic compounds and proteins and amino acids. However, hydrolysable tannins were not detected unlike in the powdered leaves.

Determination of total phenolic content: Most antioxidant activities of plant sources are derived from phenolic-type compounds. Due to their hydroxyl groups, phenols are very important plant constituents with scavenging ability. But the antioxidant effects do not necessarily always correlate with the presence of large quantities of phenolics (Slinkard and Singleton, 1977). The *Wedelia calandulaceae* Less. non-Rich. methanolic extract (MEWC) was evaluated for total phenolic content. The amount of total phenolics in this study was found to be 7.25 mg GAE g^{-1} extract. The present study did not show any correlation between phenolic content and antioxidant activity.

Total antioxidant activity determination in linoleic acid system: Thiocyanate method was used to evaluate the total antioxidant activity of the plant extract. The extract exhibited effective and powerful antioxidant activity at a concentration of 250 μg mL^{-1}. The effect of 250 μg mL^{-1} concentration of the extract on peroxidation of linoleic acid emulsion is shown in Fig. 2. The antioxidant activity of the plant extract initially was increased with an increasing time of incubation and then it showed a decrease in activity further with increasing time of incubation. The studied concentration of the extract exhibited higher antioxidant activity than 250 μg mL^{-1} concentration of α-Tocopherol but lower antioxidant activity than same concentration of BHA (Butylated hydroxyanisole). The percentage inhibition of peroxidation of the extract in linoleic acid system was found to be 61.27%. And percentage inhibition of 250 μg mL^{-1} concentration of BHA and α-Tocopherol was found as 97.02 and 30.48%, respectively.

Effect on reducing power assay: The reducing power of the extract compared to BHA and α-Tocopherol are shown in Fig. 3. In the reductive ability measurement,

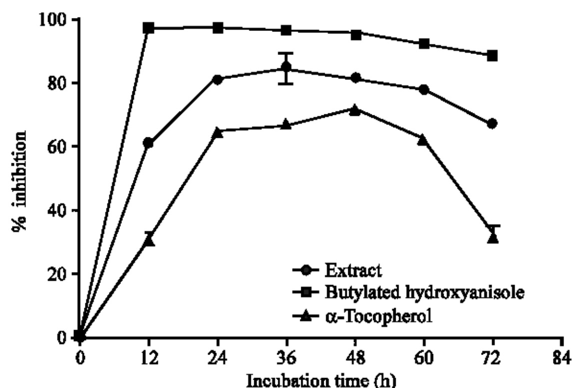

Fig. 2: Total antioxidant activity of MEWC, BHA and α-Tocopherol at 250 μg mL^{-1} concentration. Results are Mean±SEM of three parallel measurements

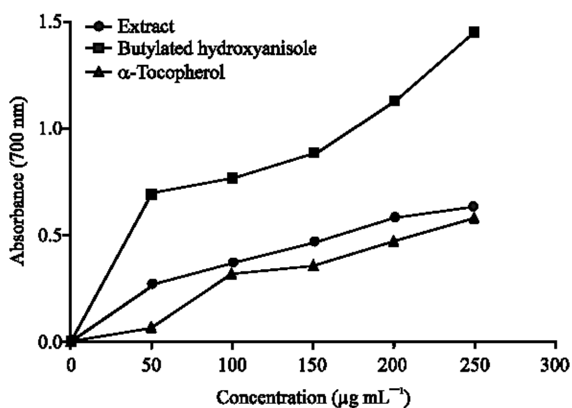

Fig. 3: Reducing power of MEWC, BHT and α-Tocopherol by spectrophotometric detection of Fe^{3+}-Fe^{2+} transformation. Results are Mean±SEM of three parallel measurements

Fe^{3+}-Fe^{2+} transformation in the presence of extract samples was investigated using the method of Oyaizu (Oyaizu, 1986). The reducing power of the extract was increased with increasing concentration of the extract. At all the studied concentrations, the extract revealed higher reducing power than α-Tocopherol but reductive capability was lower than BHA. Reducing power of the extract and standard compounds followed the order: BHA> Extract> α-Tocopherol.

Effect on DPPH radical scavenging activity: Evaluation of antioxidant activities by measuring scavenging capability of stable DPPH radical is a widely used method which requires short time compared to other methods. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.

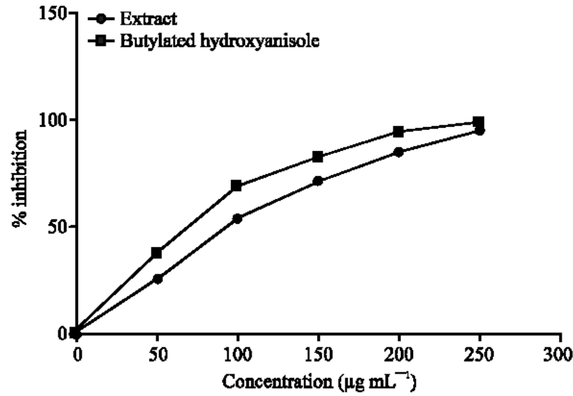

Fig. 4: Free radical scavenging activity of different concentrations of MEWC and BHA by DPPH radicals. Results are Mean±SEM of three parallel measurements

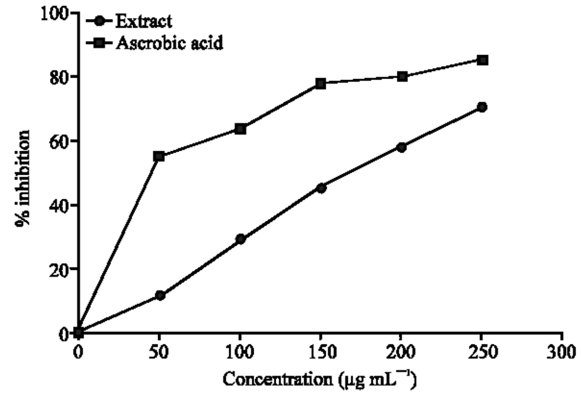

Fig. 5: Superoxide anion scavenging activity of MEWC and same doses of Ascorbic acid in PMS-NADH-NBT method. Results are Mean±SEM of three parallel measurements

Antioxidants induced decrease in absorbance at 517 nm by scavenging of DPPH radicals. Hence, DPPH is usually as a substrate to evaluate antioxidant activity of different antioxidants. In this present study, BHA was used as a standard radical scavenger. Figure 4 shows the decrease in concentration of DPPH radical due to scavenging capability of the extract and standard compound (BHA) at different studied concentrations (50-250 µg mL^{-1}). The scavenging ability of the extract on DPPH radical was found to be less than BHA. The percent DPPH scavenging effect of the extract and the standard were found to be 95.96 and 98.87%, respectively at the concentration of 250 µg mL^{-1} (Fig. 4). The results indicated the plant as a strong scavenger of DPPH radical comparable to standard BHA. The IC_{50} values of the extract and BHA were calculated using the equation obtained from linear regression analysis. The calculated IC_{50} values of the extract and the standard compound (BHA) were found to be 110.881 and 89.117µg mL^{-1}, respectively. When free radical formation exceeds the body's ability to protect itself, oxidative stress occurs and forms the biological basis of chronic condition (Jainu and Devi, 2005). Data from this present study indicate that the plant extract is powerful free radical scavenger which can reduce or reverse the damage caused by free radicals in the human body.

Effect on superoxide anion scavenging activity: Phenolic compounds particularly flavonoids and catechins are found to be important antioxidants and superoxide scavengers. The scavenging efficiency of these compounds mainly depends on the concentration of phenol and the numbers and locations of the hydroxyl groups (Ashokkumar *et al.*, 2008; Erasto *et al.*, 2007).

Table 1: Comparison of Superoxide anion scavenging activity, Nitric oxide scavenging activity and Hydrogen peroxide scavenging activity of MEWC and standard antioxidant compounds such as Ascorbic Acid at the concentration of 50 µg mL^{-1}

	Scavenging activity (%)		
	Superoxide anion	Nitric oxide	Hydrogen peroxide
Ascorbic acid	54.80±0.4407	36.50±2.435	42.54±0.4672
MEWC	11.34±0.4535	23.95±0.2762	29.81±0.7410

Superoxide anion is a highly toxic species and generated by different biological reactions in the physiological system. In the present study, the decrease in absorbance at 590 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Table 1 shows superoxide anion scavenging activity of the extract and standard antioxidant compound at concentration of 50 µg mL^{-1}. Figure 5 shows the increase in percentage inhibition of superoxide radical generation with increasing in concentration of the extract and standard compound (Ascorbic acid). The plant extract showed good superoxide radical scavenging activity but the scavenging activity of the extract was found to be lower than the standard ascorbic acid. The percentage inhibition by the extract and ascorbic acid were found as 70.17 and 84.80%, respectively.

Effect on nitric oxide scavenging activity: Nitric oxide or Reactive Nitrogen Species (RNS) are very reactive compounds which can change pathologically the structural and functional behaviour of many cellular components (Ashokkumar *et al.*, 2008). In this present experimental design, scavenging of nitric oxide by the extract was evaluated by observing the reduction of linear time-dependent nitrite production in the sodium

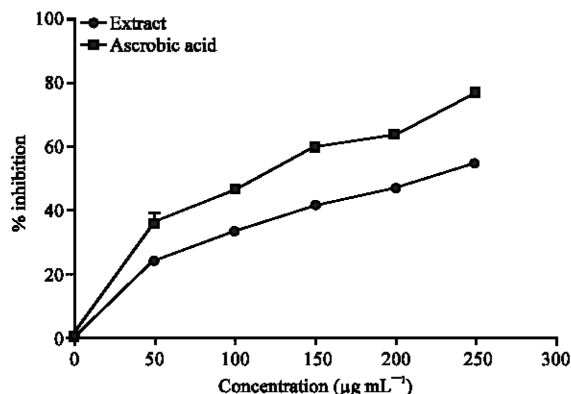

Fig. 6: Percentage inhibition of nitric oxide radical by MEWC and Ascorbic acid. Results are Mean±SEM of three parallel measurements

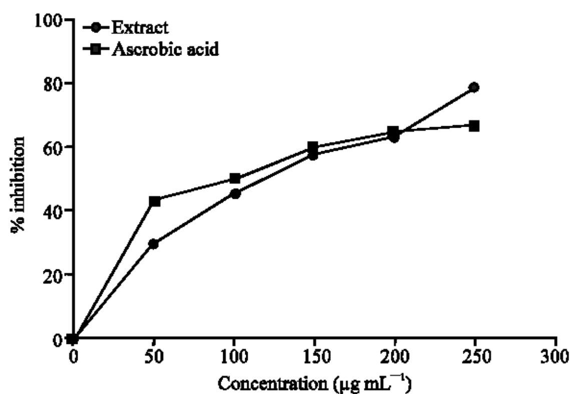

Fig. 7: Hydrogen peroxide activity of different concentrations of MEWC and Ascorbic acid. Results are Mean±SEM of three parallel measurements

nitroprusside-PBS system. Figure 6 shows the concentration dependent scavenging of nitric oxide by the extract and standard compound (Ascorbic acid). Table 1 shows nitric oxide scavenging activity of the extract and standard antioxidant compound at concentration of 50 μg mL^{-1}. It is observed that all the concentrations of the plant extract (50-250 μg mL^{-1}) are likely to have the nitric oxide scavenging activity. The nitric oxide scavenging activity of the extract was found to be lower than ascorbic acid. The percentage inhibition of nitric oxide by the extract and standard was calculated as 54.61 and 76.76% at the concentration of 250 μg mL^{-1}, respectively. The IC_{50} values were calculated from the equations obtained from linear regression analysis of the data and found as 207.615 and 135.531 μg mL^{-1} for the extract and ascorbic acid, respectively.

Effect on Hydrogen peroxide scavenging activity: Hydrogen peroxide gives rise to hydroxyl radicals. Removing hydroxyl radicals ($^{\bullet}OH$) is very essential for the protection of living system as they react with most biomolecules and other cellular components to cause tissue damage leading to cell death (Dhully *et al*., 1993; Reddy *et al*., 2010). In this present study, ability of the plant extract at different concentrations (50-250 μg mL^{-1}) to scavenge H_2O_2 was evaluated. Figure 7 shows the percentage H_2O_2 scavenging effect by different concentrations of the plant extract and standard compound (ascorbic acid). Table 1 shows hydrogen peroxide scavenging activity of the extract and standard antioxidant compound at concentration of 50 μg mL^{-1}. The plant extract showed good H_2O_2 scavenging ability when compared to standard compound. The percentage H_2O_2 scavenging effect by the same concentration (50 μg mL^{-1}) of the plant extract and ascorbic acid was found as 29.81 and 42.54%, respectively. But 250 μg mL^{-1} concentration of the extract showed higher percentage scavenging effect than the same concentration of ascorbic acid.

CONCLUSION

Based upon the results from different *in vitro* antioxidants models it is evident that MEWC has an effective and considerable antioxidant profile. The possible mechanism of action for these different antioxidant activities includes hydrogen-donating ability, reducing ability, scavenging ability of superoxide anion radical, nitric oxide, hydrogen peroxide and DPPH radical. The presence of different phytoconstituent in MEWC may be responsible for the antioxidant mechanisms. The preliminary phytochemical investigation and previous phytochemical works also suggested several phytoconstituent with potential antioxidant activity. It is therefore concluded that *Wedelia calandulaceae* can be a good source of natural antioxidants. The results of this study are supportive of the usefulness of this plant in Indian system of medicine and also its use as functional food.

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