

## RESEARCH ARTICLE

# *In vitro* antioxidant activity and selenium content of some important wild edible plants from arid zone

■ Mala Rathore

### SUMMARY

Inverse relationship is seen between dietary intake of antioxidant-rich foods and incidence of number of human diseases. They play an important role in chemoprevention of diseases *viz.*, cancer, AIDS, arthritis, osteoporosis, CNS injury. There are a number of local foods from arid zone of Rajasthan used traditionally which may have potential positive effects on health but antioxidant properties of edible wild plants have not been determined. Present study is an attempt to explore some of the promising wild edible plant resources of arid zone of Rajasthan for their antioxidant potential by studying their DPPH activity and selenium content. The antioxidant activity of the selected plant species determined by DPPH assay was found to vary from 38.99 to 89.48%. The selenium content in the selected wild edible plants varied from 2.53 µg/g (*Grewia tenax*) to 3.49 µg/g (*Leptadenia reticulata*) on dry wt basis. The antioxidant values and selenium content values suggest that these plants can be used as supplementary food. Studies are however needed to study the effect of cooking, processing, geographical variation and *in vivo* trials.

Key Words : Antioxidant activity, Selenium content, Some important wild edible plants

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Achieving an optimal or maximal state of nutrition and health is becoming a matter of much concern. There is an increased awareness of the importance of food supplements as a part of daily food intake. People are becoming more conscious about their well being and are shifting to nutraceutical products to

prevent the onset of any chronic diseases. Antioxidants are most common type of nutraceuticals (<http://www.state.oh.us/age/>).

Antioxidants work as free radical scavengers in living organisms and block their harmful effect. Inverse relationship is seen between dietary intake of antioxidant-rich foods and incidence of number of human diseases. They play an important role in chemoprevention of diseases *viz.*, cancer, AIDS, arthritis, osteoporosis, CNS injury etc. (Rajasekaran *et al.*, 2008; Dureja, 2003 and

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Due to depletion of immune system natural antioxidants in different maladies, consuming antioxidants is necessary. Antioxidant activity has been reported in a few edible fruits and vegetables (Kiselov, 2005; Odukoya *et al.*, 2005; Aline Lamien-Meda, 2008 and Odukoya *et al.*, 2007). Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves (Iqbal and Bhangar, 2006) has been studied. There are a number of local foods from arid zone of Rajasthan used traditionally which may have potential positive effects on health (Rathore and Meena, 2004). There is, however, meagre literature concerning antioxidant properties of edible wild plants (Jain *et al.*, 2009). Antioxidant activity of some medicinally important arid zone plants has been studied by Singh *et al.* (2009). Present study is an attempt to explore some of the promising wild edible plant resources of arid zone of Rajasthan for their antioxidant potential by studying their DPPH activity and selenium content.

## MATERIAL AND METHODS

All the chemicals and standards used were of Merck and Sigma Make.

### Collection of plant material :

Reconnaissance survey was carried out in various parts of Rajasthan as finalized through interaction with local people, forest Department officials, University professors places for collection of selected plants. Plant materials *viz.*, leaf, fruit and tuber samples of selected plant species were collected from atleast 3-4 different locations from various districts in Rajasthan. *Leptadenia reticulata* was found under irrigated conditions. The collected samples were cleaned, washed and dried in shade for 12-15 days and then stored in airtight containers with labelling at room temperature. These were ground in a / mixer grinder to a fine homogeneous powder before analysis.

### Measurement antioxidant activity (DPPH Assay):

*Chemicals required :*

- 1,1-diphenyl-2-picryl hydrazyl (DPPH) reagent.
- Ascorbic acid.

### Extract preparation :

The powdered material was successively extracted in methanol in a soxhlet apparatus by hot percolation

technique. The extracts were then concentrated by evaporating the solvent under reduced pressure for determination of antioxidant activity (1, 1-Diphenyl-2-picryl-hydrazyl (DPPH•) free radical scavenging activity).

### DPPH assay :

The free radical scavenging activity of DPPH was determined by the method of Gadov *et al.*, 1997, the colour disappearance of the stable free radical, DPPH• is monitored at a 517 nm wavelength in the presence of the sample. In its radical form, DPPH• absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorbance decreases.

Methanolic 0.1 mM solution of DPPH• (0.399 mg of DPPH and dissolve in 50 ml of methanol) was prepared and 1ml of this solution was added to 3 ml plant sample solution (stock solution was prepared by dissolving in methanol) at different concentration (25-250 µg/ml). The absorbance was measured at 517 nm after 30 min. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Blank reading is also taken. The DPPH• radical scavenging activity was calculated according to the following eq.

$$\text{DPPH radical scavenging activity (\%)} = \left[ \frac{A_0 - A_1}{A_0} \right] \times 100.$$

where,

$A_0$  = Absorbance of the DPPH•,

$A_1$  = Absorbance of the extract in DPPH• solution.

### Selenium determination using ICPMS :

*Chemicals required :*

- HNO<sub>3</sub> (Suprapure) 65%
- HCl (30%)
- HF (40%)
- Boric Acid (4%)
- Standard 77Se
- deionised water 0.55µS/cm.

### Preparation of sample :

0.125 g of plant sample was taken in the digestion tube (Xpress) 55 ml capacity and 4.5 ml of HNO<sub>3</sub> (Suprapure) 65% , 1 ml of HCl (30%) and 1 ml of HF (40%) was added and kept in the rotator stand and digested in CEM MARS 6 Microwave Digester under the following conditions :

- Magnetron power - 800W
- Temp 180 °C,
- Ramp time -20 min

Hold time -35 min  
Cooling time- 15 min

After digestion sample (HF) was neutralized using 15 ml boric acid (4%) and again kept in digester and neutralized in MARS 6 Digester with following conditions:

Magnetron power - 800W  
Temp 180 °C,  
Ramptime-10 min  
Hold time -15 min  
Cooling time-15 min

After neutralization samples were removed and then filtered by PES 0.45 micron filter prior to being analyzed. Samples were made upto 50 ml with 1% deionised water 0,55µS/cm, the solution was filtered using syringe filter (2.5mm, 0.45 µPS) and analysed in Thermo-Fischer ICMS Qc tuned in standard mode at following conditions:

Flame power = 10548.6 watt  
Sampling depth = 5  
Detector voltage (Counting feedback) = 1075 V  
Plasma cooling water flow = 3.82 lit/min  
Exhaust flow=0.654 lit/min  
Nebulizer supply pressure= 3.688 pascal  
Argon flow = 12-14 l/min  
Peristaltic pump = 40 rpm  
Uptake time= 30 Secs  
Wash time = 30 secs

Standard used - 77Se  
Std 1-0.505 ppm  
Std 2- 0.1 ppm  
Std 3- 1 ppm  
Std of 0.2 and 0.5 omitted  
 $R^2 = 0.99$   
Relative error = 4.573%

## RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

### Antioxidant activity

Among the various methods used for determining

antioxidant activity, DPPH free radical scavenging method predicts antioxidant activities in relatively short time. In this assay, violet colour DPPH solution is reduced to yellow coloured product, diphenylpicryl hydrazine, by addition of the extract in a concentration dependent manner. The antioxidant activity of the selected plant species determined through this method varied from 38.99 to 89.48%. The activity of ascorbic acid was found as 96.63%. Average maximum antioxidant activity (89.48%) has been found in *Calligonum polygonoides* pods. Higher activity was of samples from Jaisalmer (Nachna) region (95.1%). *Cassia tora* leaves also exhibited second highest activity (84.58%) maximum being from Udaipur region. *Ceropegia bulbosa* and *Cordia gharaf* exhibited average antioxidant activity, 44.84% and 43.02%, respectively.  $IC_{50}$  values were calculated for these samples were found to vary from 10.2 to 99.77%.

### Selenium content :

Selenium is an essential nutrient required in food and works as an antioxidant. It plays an important role in many metabolic pathways such as thyroid hormone metabolism and antioxidant defense systems (Zimmerm *et al.*, 2015) and is linked with some serious conditions like cancer, cardiovascular and inflammatory diseases (Weekly and Harris, 2013). Selenium is a cofactor of the enzyme glutathione peroxidase and a catalyzer of the reduction of peroxides, which can damage cells and tissues (Rotruck *et al.*, 1973). The main source of selenium in human beings is their diet and thus it has important implications for human nutrition and health .

The amount of selenium in any food varies greatly depending on the quality of the soil on which the food was produced. It is required in very small amount only. The Food and Nutrition Board of the Institute of Medicine (USA) has proposed a Recommended Dietary Allowance (RDA) of 55 µg Se day-1 for adults and a tolerable upper intake of 400 µg Se day-1 (Krinsky *et al.*, 2000). According to the Institute of Medicine, Food and Nutrition Board (2000), the maximum limit of Se is 400 µg/day over which negative selenium effects are expected (Arthur, 1991).

The knowledge of total selenium content present in a particular food or dietary supplements is necessary. The selenium content in the selected wild edible plants from arid region was determined and it varied from 2.53 µg/g (*Grewia tenax*) to 3.49 µg/g (*Leptadenia reticulata*) on dry wt basis which is well within the intake limit. The selenium content of Papaya, pineapple, guava,

**Table 1 : DPPH scavenging activity and IC<sub>50</sub> values of selected edible plants**

Species (Part Used)	Place	% Scavenging activity (100µl/ml Solution)	Average scavenging activity	IC <sub>50</sub> value
<i>Cassia tora</i> (Leaves)	Banswara	79.73	84.58	27.1
	Udaipur	90.09		19.09
	Beda	86.03		13.6
	Dungarpur	82.47		20.4
<i>Grewia tenax</i> (Fruits)	Kailana	37.76	38.99	93.67
	Jaswantpura	40.22		88.56
<i>Ceropegia bulbosa</i> (Tubers)	Beda	42.12	44.84	75.12
	Udaipur	33.96		93.955
	Jaswantpura	58.44		65.14
<i>Leptadenia pyrotechnica</i> (Pods)	Barmer	66.86	62.28	56.21
	Bikaner	61.79		62.31
	Nagaur	58.2		75.65
<i>Haloxylon salicornicum</i> (Seeds)	Nachna	41.49	52.35	71.46
	Siyamber	42.98		80.35
	Lathi	66.43		71.42
	Phalodi	58.50		79.53
<i>Calligonum polygonoides</i> (Flower buds)	Bikaner	78.47	89.48	19.8
	Barmer	94.88		10.6
	Jaisalmer	95.10		10.2
<i>Cordia gharaf</i> (Fruits)	Jodhpur	37.09	43.0	99.77
	Jaswantpura	49.05		92.83
<i>Leptadenia reticulata</i> (Pods)	Pali	68.8	74.67	59.29
	Jodhpur	80.55		36.14
Ascorbic acid	Standard	96.63	96.63	14.83

**Table 2 : Selenium content of selected edible species**

Plant species	Part used	Place of collection	Moisture content (%)	Selenium content µg/g dry wt basis
<i>Cassia tora</i>	Leaves	Pali	70.55	2.89
<i>Calligonum polygonoides</i>	Flower buds	Bikaner	62.7	3.30
<i>Leptadenia pyrotechnica</i>	Pods	Bikaner	80.9	3.25
<i>Leptadenia reticulata</i>	Pods	Jodhpur	85	3.49
<i>Ceropegia bulbosa</i>	Tubers	Udaipur	84.54	3.07
<i>Cordia gharaf</i>	fruits	Jodhpur	68.49	2.89
<i>Grewia tenax</i>	fruits	Jodhpur	67.92%	2.53
<i>Haloxylon salicornicum</i>	Seeds	Phalodi	16.7	2.96

mango and raspberry fruits ranges from 60 µg/100g to 1600 µg/100g (Raymond, 1983).

### Conclusion:

Methanolic extracts of selected wild edible plants showed that these have good antioxidant activity as measured by DPPH antioxidant assay. Thus, consumption

of these wild plants could contribute to providing benefits such as protecting against oxidative damage under different conditions. The antioxidant values and selenium content suggests that these plants can be used as supplementary food but further studies on effect of cooking, processing, geographical variation and in vivo trials are needed before advocating them to general

public.

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