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Research Article

Antioxidative potential of defatted meal from exotic collections of Indian mustard (*Brassica juncea*)

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Abstract
The present study was directed to evaluate antioxidant potential of methanolic extract from defatted meal of twenty Exotic collections (EC) of *Brassica juncea* for selection of desirable breeding germplasm having functional value in term of antioxidative potential. Being antioxidative in nature, *B. juncea* may be used as an alternative in place of synthetic ones. Total antioxidant activity and phenol content was observed in the range 14.204 to 26.973 mg/g and 5.39 to 7.47 mg/g, respectively. In term of IC_{50} value Hydroxyl radical, nitric oxide & superoxide radical scavenging was maximum in EC564649 where as DPPH radical scavenging was maximum in EC552583 (YS). Metal chelating activity was maximum in EC564641.

Key words
Exotic collections, *Brassica juncea*, Antioxidative and phenol content

Introduction

Brassica juncea is an important oil seed crop of family *Brassicaceae* which comprises of 400 genus and 4,000 species (Dal Prá *et al.* 2013). High levels of antioxidant metabolites in *Brassica* vegetables provide beneficial health effects (Ferreres *et al.* 2007). Natural health products and food obtained from plant have some common biological properties like antioxidant activity, capacity of scavenging electrophiles, ability of scavenging active oxygen species, inhibition of nitrosation, metals chelating ability, the capability of production of hydrogen peroxide in the presence of certain metals, and the potential to alter certain enzyme activities (Terpinc *et al.* 2012). Antioxidants showed their contribution both to the first and second defense lines against oxidative stress and may therefore prevent chronic diseases, such as cardiovascular disease, cancer, and diabetes (Podsędek, 2007). In recent times, mustard meal was found to be rich in different bioactive substances with health promoting properties and rich in anti-oxidative factors and good source of dietary minerals (Punetha *et al.*, 2015 & 2017), which can be utilized for preparation of functional food as well as for selection of breeding material having desirable antioxidative level. Keeping this fact in mind present investigation focused to evaluate the antioxidative potential of Exotic Collections (EC) of Indian mustard.

Material and Methods

Source of plant material: The seed samples of 20 EC genotypes were collected from the Crop Research Centre, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar after proper validation by Plant breeder.

Preparation of Defatted mustard meal: Crushed seeds of mustard were packed in Muslin cloth. This thimble was placed in Soxhlet apparatus and defatting was done at 60° C using petroleum ether. After 8-9 times of repeated extraction Oil was extracted by evaporating the petroleum ether extract and the defatted meal obtained was used further for antioxidative potential.

Preparation of methanolic extract: **D**efatted meal (0.1 g) was mixed thoroughly with 2.0 ml of 80% methanolic solution. After centrifugation at 3000 rpm for 10 minutes methanolic extract was used for further analysis.

Determination of Total Phenolic Content: Determination of Total phenolic content follows the principle that phenolic components of mustard are oxidised by phosphomolybdate. Phosphotungstic acid on reduction get converted into blue colored oxide of tungsten and molybdenum resulting in formation of blue colored comlex which was estimated as gallic acid equivalent by Folin-Ciocalteau method. Absorbance was measured at 725 nm.

Total anti-oxidant content: It was estimated by the method of Prieto *et al.* (1999) with slight modification. The test was based upon the reduction of Mo (VI) to Mo (V) by the extract. 0.1 ml of methanolic extract was taken in a test tube and to it 1.0 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4.0 mM ammonium molybdate) were added. The sample was measured at 695 nm against a blank. The anti-oxidant activity was expressed relative to that of ascorbic acid.

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DPPH (1,1-diphenyl-2-picrylhydrazyl) Free Radical-Scavenging Activity: The DPPH radical-scavenging activity of methanolic extracts was determined, following the procedure described by Espin, (2000). Different concentrations ranging between 200 µg/ml to 800 µg/ml of methanolic extract of defatted meal were taken in different test tubes and to each test tube 5.0 ml of 0.004% methanolic solution of DPPH was added. All the samples were placed in the dark for 30 minutes and the discoloration in samples was read at 517 nm using UV-spectrophotometer.The percentage Inhibition of free radical by DPPH (IC%) was calculated by the following equation.

$$\%\ \text{Inhibition Capacity(IC)} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \times 100$$

Metal chelating Activity: To the aliquot of the methanolic extract of the test substance of varying concentrations (200-800µg/ml), 100 µl of 2.0 mM $FeCl_2.4H_2O$ solution was added. Reaction was commenced by adding 5.0 mM ferrozine solution (200 µl) followed by addition of methanol to make up the volume to 5.0 ml. On mixing properly the solution were allowed to incubate at room temperature for about 10 minutes. At 545 nm absorbance was recorded. EDTA was taken as the chelating standard. Chelating activity was calculated using equation given below:

$$\text{IC \%} = \frac{Ao - At}{Ao} \text{ x } 100$$

Superoxide anion scavenging activity: It was determined by the method of Nishimiki *et al.* (1972) with little variation. To the aliquot of the methanolic extract of the test substance of varying concentrations (200-800 µg/ml), 1 ml of Nitrobluetetrazolium solution (100 mmol/l phosphate buffer, pH 7.4) was added followed by addition of NADH (468 µmol in 100 Mm/l phosphate buffer, pH 7.4). Reaction was initiated by addition of 100 µl of Phenazinemethosulphate solution (60 mM of 100 mM/l phosphate buffer, pH 7.4). The percent inhibition was calculated by equation given below.

$$\%\ \text{Superoxide scavenging activity (IC \%)} = \frac{Ao - At}{Ao} \text{ X } 100$$

Nitric oxide radical scavenging Activity: The activity was evaluated using the method given by Naskar *et al.* (2010). To the aliquot of the methanolic extract of the test substance of varying concentrations (200-800 µg/ml), 2 ml of Sodium nitroprusside (SNP) (10mM) in phosphate buffer saline (PBS) pH 7.4 was added, mixed well and incubated for about two and a half hours at 25° C. To this mixture 1 ml of Griess reagent (1% sulphanilamide, 0.1% naphthylehtylene diamine dichloride and 2 ml orthophosphoric acid) was added. As a result pink color was obtained and absorbance read at 546 nm keeping Ascorbic acid as standard. The percent inhibition was calculated by equation given below.

$$\%\ \text{Nitric oxide scavenging activity (IC \%)} = \frac{Ao - At}{Ao} \text{ X } 100$$

Hydroxyl radical scavenging activity: In methanolic extracts of mustard meal Hydroxyl radical scavenging activity was determined by the method described by Olabinri *et al.* (2010) with little variations. To the aliquot of the methanolic extract of the test substance of varying concentrations (200-800 µg/mL), 60 µl of $FeSO_4.7H_2O$ (1mM) was added to 90 µl of aqueous 1,10 Phenanthroline (1mM), into this mixture 2.4 ml of 0.2 M Phosphate buffer (pH 7.8) was added followed by addition of 150 µl of Hydrogen peroxide (0.17 mM). Absorbance of various test samples was read at 560 nm against blank after 5 minutes. Ascorbic acid was taken as standard. The percent inhibition was calculated by equation given below.

$$\%\ \text{Hydroxyl radical scavenging activity (IC \%)} = \frac{Ao - At}{Ao} \text{ X } 100$$

Statistical analysis: Data obtained in all experiments were in triplet and were mean ± SE subjected to one way ANOVA by using SPSS 16. Means were separated by Duncan multiple range test (Duncan, 1955).

Results and Discussion

Total Phenol Content (TPC): The highest total phenol content was observed in EC 564641 (7.47±0.38 mg/g gallic acid equivalent) followed by EC 552583(YS) (7.24±0.21 mg/g), EC 552582 (7.17±0.29 mg/g) where as minimum was recorded in EC 552581(YS) (5.39±0.37 mg/g) (Table 1). The observations revealed that phenol content varied significantly among the different *Brassica* genotypes.

Total anti-oxidants content: It was observed to be minimum in EC 552581(BS) (14.204mg/g) and maximum in EC 399302 (26.973 mg/g). The total anti-oxidants content varied significantly among the *Brassica* genotypes (Table 1). In present study, the total antioxidative activity in defatted meal of *B. juncea* might be due to the presence of phenols.

DPPH radical scavenging activity: The ability of mustard meal to quench reactive species by hydrogen donation were evaluated by measuring the DPPH radical scavenging activity of different concentrations of methanol extracts (Fig.1 and Table 2). The activity in terms of their IC_{50} was maximum in EC 552583(YS) (IC_{50} = 301.82 ± 35.03 µg/ml) and minimum in EC 564649 (IC_{50} = 416.71 ± 18.52 µg/ml).

Metal chelating: The indication of higher chelation was expressed in terms of IC_{50} values. The order of higher antioxidant potential was seen in order EC 564641 (IC_{50} = 115.41 ± 11.97 µg/ml) > EC552578 (IC_{50} = 144.96 ± 30.65 µg/ml) >EC

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399296 (IC_{50} = 150 ± 5 µg/ml) > EC 552577 (IC_{50} = 189.97 ± 26.59 µg/ml). The percent chelating activity and IC_{50} values of methanolic extract of mustard meal assayed for Fe^{2+} chelating activity are shown in figure 2 and table 2. Minimum IC_{50} value indicated maximum chelating activity.

The superoxide scavenging ability: The method is based on the fact that anion (O^{2-}) was generated by reduction of yellow dye (NBT^{2+}) to produce the blue formazan by xanthine oxidase, *in vitro*. The order of Superoxide anion scavenging activity was found to be greater for EC 564649 with a lower IC_{50} value (IC_{50} = 321.73±18.39 µg/ml) followed by EC564646 (IC_{50} = 446.56±14.43 µg/ml) > EC 552573 (IC_{50} = 584.14±19.68 µg/ml) (Fig. 3 and Table 2).

Nitric oxide (NO) radical activity: The highest antioxidant ability in terms of IC50 was recorded in EC 564649 (IC_{50} = 125.97±4.90 µg/ml) followed by EC564646 (IC_{50} = 269.65±13.69 µg/ml) and EC 552573 (IC_{50} = 297.36±13.38 µg/ml). The detailed experimental data reported in figure 4 and table 2.

Hydroxy radical activity: The scavenging avtivity of methanolic extracts from defatted meal of 20 EC genotypes towards OH free radical in comparison to the standard antioxidant ascorbic acid reported in fig. 5 and table 2. In terms of % inhibition and IC_{50} values the dose dependent OH radical scavenging activity in all the genotypes of EC genotypes were found in order of EC 564649 (IC_{50} = 125.97±4.90 µg/ml) > EC552578 (IC_{50} = 279.20±17.03 µg/ml) > EC564646 (IC_{50} = 297.65±10.91 µg/ml) > EC 552573 (IC_{50} = 404.76±11.11 µg/ml). The minimum IC_{50} values indicated higher scavenging activity thus revealing good antioxidant activity of samples under investigation.

Conclusion

The antioxidant properties of phenolic compounds are due to their scavenging ability, primarily because of the presence of hydroxyl groups. Activity of these compounds may be related to their ability to chelate metals and inhibit oxidative damage. The defatted meal contained different anti-oxidants, with important biological activities. Findings of present study may be employed for determining the nutritional status and quality character of *Brassica juncea* and may be used to establish their potential towards expansion of new and better cultivars. Moreover the defatted meal having higher level of anti-oxidants can be utilized for the preparation of functional food for animal and human consumption.

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References

DalPra, V., Jardim, N.S., Dolwitsch, C.B., Mazutti, M.A., Viana,C., Bohrer, D., Nascimento, P.C., Carvalho, L.M. de, Silva, M.B. da, Carvalho, C.A.de and Rosa, M.B. da. 2013. A review of influence of environment and process parameters on glucosinolate-myrosinase system from *Brassica*. *JAPS*, **3**(08): 121-128.

Duncan, D. B. 1955. Multiple range and multiple F tests. *Biometrics*, **11**: 1–42.

Espin, J.C., Rivas, C.S., Wichers, H.J. 2000. Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-1-picrylhydrazyl radical. *J. Agric. Food Chem.*, **48**: 648–656.

Ferreres, F., Sousa, C., Valenta, P., Seabra, R.M., Pereira, J.A. and Andrade, P.B. 2007. Tronchuda cabbage (*Brassica oleracea* L. var. *costata* DC) seeds: Phytochemical characterization and antioxidant potential. *Food Chem.*, **101**: 549–558.

Naskar, S., Islam, A., Mazumder, U.K., Saha, P., Haldar, P.K. and Gupta, M. 2010. *In Vitro* and *In Vivo* Antioxidant Potential of Hydromethanolic Extract of Phoenix dactylifera Fruits. *J. Sci. Res.*, **2**(1): 144-157.

Nishimiki, M., Rao, N.A. and Yagi, K. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem. Biophys. Res. Commun.*, **46**: 849–853.

Olabinri, B.M., Adetutu, A., Olaleye, M.T., Oluwafunsho, B.O. and Oyeniyi, O.O. 2013. A study of the antioxidative potentials of acetone and aqueous extracts of *Parkia biglobosa* and *Tetracarpidium conophorum* stem barks *in vitro*. *Int. J. Med. Med. Sci.*, **5**(8): 368-373.

Podsędek, A. 2007. Natural antioxidants and antioxidant capacity of *Brassica* vegetables: A review. *LWT - Food Sci and Technol.*, **40**(1): 1-11.

Prieto, P., Pineda, M. and Aguilar, M. 1999. Spectro photometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal. Biochem.* **269**: 337-341.

Punetha, H., Chandra, M. Bhutia, Tripathi, S. and Prakash, O. 2015. Antioxidative properties and mineral composition of defatted meal of oileferous *Brassica* germplasm. *TBAP*, **5**: 43-45

Punetha, H., Sagar, Heena, Panwar, Swati, Pant, Usha and Prakash, Om. 2017. Study on phytochemicals and antioxidative capacity of selected genotypes of *Brassica juncea*. *Res. Environ. Life Sci.* **10**(4): 373-377.

Terpinc, P., Ceh, B., Ulri, N.P. Abramovic, H. 2012. Studies of the correlation between antioxidant properties and the total phenolic content of different oil cake extracts. *Ind Crop Prod.*, **39**: 210– 217.

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Table 1. Total phenol content (TPC) and Total antioxidant content in exotic collections of Indian mustard

Sl. No.	Genotypes	Total phenolic content (mg/g)	Total antioxidant (mg/g)
1	EC 552583(BS)	6.37±0.23^{cde}	17.198±0.025^{d,e}
2	EC552578	6.11±0.19^{bcd}	16.062±0.048^{b,c}
3	EC399299	6.16±0.37^{bcd}	18.173±0.540^{e,f}
4	EC552584	5.63±0.036^{bc}	17.580±0.020^{c,d,e}
5	EC564646	5.78±0.57^{abc}	20.323±0.055^{g}
6	EC552582	7.17±0.29^{h}	17.851±0.010^{e,f}
7	EC 552573	6.22±0.01^{bcd}	17.020±0.520^{c,d}
8	EC 399296	6.25±0.17^{bcd}	17.763±0.179^{c,d,e}
9	EC 552583(YS)	7.24±0.21^{h}	21.240±0.199^{g}
10	EC 552581(YS)	5.39±0.37^{a}	17.280±0.020^{c,d,e}
11	EC 552581(BS)	5.90±0.57^{abcd}	14.204±0.070^{a}
12	EC 564648	6.19±0.47^{bcd}	23.280±0.020^{h}
13	EC 399301	7.09±0.08^{gh}	17.786±0.015^{e,f}
14	EC 564640	6.97±0.35^{fgh}	22.892±0.011^{h}
15	EC 552577	6.26±0.21^{bcd}	23.851±0.010^{h}
16	EC 39912	6.32±0.02^{cd}	14.460±0.01^{a}
17	EC 552579	6.52±0.34^{def}	20.663±0.015^{g}
18	EC 564641	7.47±0.38^{h}	19.470±0.010^{f}
19	EC 564649	6.32±0.21^{cd}	16.316±0.015^{b}
20	EC 399302	5.99±0.74^{abcd}	26.973±0.011^{i}

The values shown are mean values ± standard deviation (n=3) and the values with same superscript are not statistically different at (P≤0.05)

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Table 2. IC_{50} of DPPH radical scavenging, Metal chelating, Superoxide radical scavenging, Nitric oxide radical scavenging and Hydroxide radical scavenging activity of defatted meal of exotic collections of Indian mustard

S. No.	Genotypes	Mean IC_{50} value (µg/ml) ± SD				
		DPPH radical activity	Metal Chelating activity	Superoxide radical activity	nitric oxide radical activity	Hydroxide radical activity
1.	EC 552583 (BS)	571.56±15.77^{c}	509.00±13.59^{e}	729.39±11.32^{f}	605.75±10.46^{g}	614.73±13.54^{e}
2.	EC552578	554.09±38.11^{c}	144.96±30.65^{a}	657.51±20.93^{e}	392.64±22.60^{c}	279.20±17.03^{a,b}
3.	EC399299	928.92±18.47^{f}	462.25±6.29^{d}	1141.42±19.12^{l}	1082.95±27.03^{n}	984.66±3.73^{l}
4.	EC552584	561.53±1.99^{c}	808.91±53.94^{j}	1035.30±27.85^{k}	828.15±15.69^{k}	678.00±13.02^{f}
5.	EC564646	399.44±8.97^{b}	666.01±16.82^{h}	446.56±14.43^{b}	269.65±13.69^{b}	297±10.91^{b}
6.	EC552582	976.18±24.67^{f,g}	662.36±2.74^{h}	747.54±13.18^{f}	556.47±11.71^{f}	617.15±17.12^{c}
7.	EC 552573	597.59±23.34^{c,d}	778.47±24.84^{j}	584.14±19.68^{d}	297.36±13.38^{b}	404.76±11.11^{c}
8.	EC 399296	854.46±12.21^{e}	150±5^{a}	1157.15±23.31^{l}	1212.46±14.93^{p}	1021.78±7.61^{m}
9.	EC 552583 (YS)	301.82±35.03^{a}	1000.73±31.88^{k}	852.67±8.63^{h}	712.83±9.16^{i}	790.96±22.17^{i}
10.	EC 552581 (YS)	1265.56±70.39^{i}	733.75±23.96^{i}	1487.94±32.94^{n}	1036.83±30.53^{m}	938.94±18.51^{j,k}
11.	EC 552581 (BS)	1126.07±121.38^{h}	680.14±12.09^{h}	741.65±20.33^{f}	828.29±16.21^{k}	730.03±13.11^{g}
12.	EC 564648	576.36±26.65^{c}	598.02±20.76^{f,g}	531.30±12.65^{c}	496.23±10.42^{d}	622.84±11.32^{e}
13.	EC 399301	1032.07±33.80^{g}	601.12±18.44^{g}	970.76±36.40^{g}	980.77±18.68^{l}	931.18±28.26^{j}
14.	EC 564640	1002.18±21.33^{g}	557.94±28.72^{f}	1334.87±13.72^{m}	1282.54±10.83^{q}	1058.23±12.56^{n}
15.	EC 552577	658.79±16.99^{d}	189.97±26.59^{b}	715.14±13.61^{f}	588.92±10.65^{g}	532.51±16.86^{d}
16.	EC 39912	548.85±4.43^{c}	695.63±18.55^{h,i}	783.25±13.25^{g}	682.90±14.46^{h}	638.91±24.62^{e}
17.	EC 552579	1006.54±26.96^{g}	375.06±15.36^{c}	896.32±4.85^{i}	1159.95±37.01^{o}	962.65±17.70^{k,l}
18.	EC 564641	597.64±18.28^{c,d}	115.41±11.97^{a}	897.15±19.96^{i}	758.46±11.50^{g}	761±13.380^{h}
19.	EC 564649	416.71±18.52^{b}	598.58±25.76^{f,g}	321.73±18.39^{a}	125.97±4.90^{a}	252.64±21.98^{a}
20.	EC 399302	800.49±59.39^{e}	600.63±24.90^{g}	813.87±24.60^{g}	527.52±7.33^{e}	753.13±25.98^{g,h}

Data shown are mean values ± standard deviation (n=3).
The values with same superscript are not significantly different at (P≤0.05).

Fig. 1. DPPH radical scavenging activity in defatted meal of exotic collections of Indian mustard

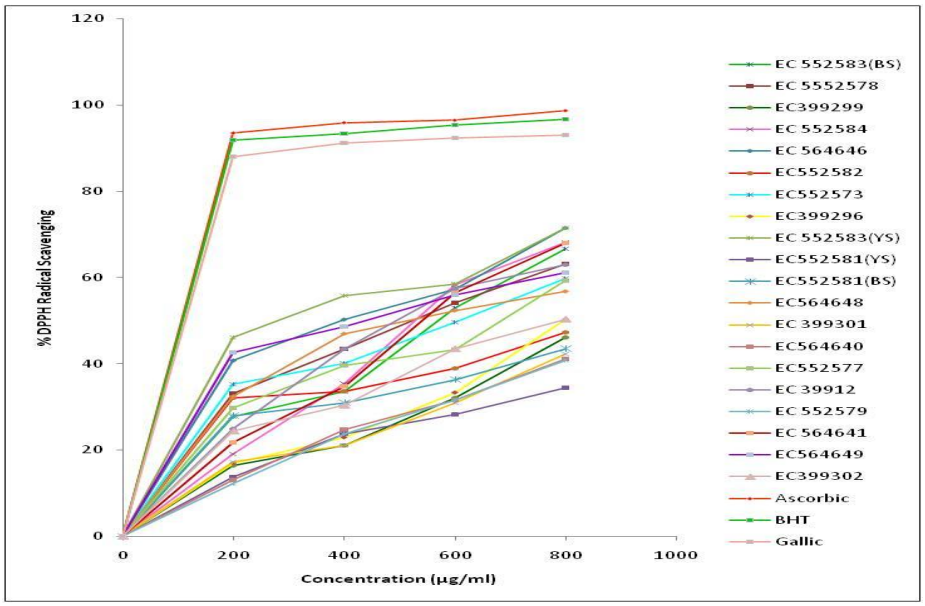

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Fig. 2. Metal chelating activity in defatted meal of exotic collections of Indian mustard

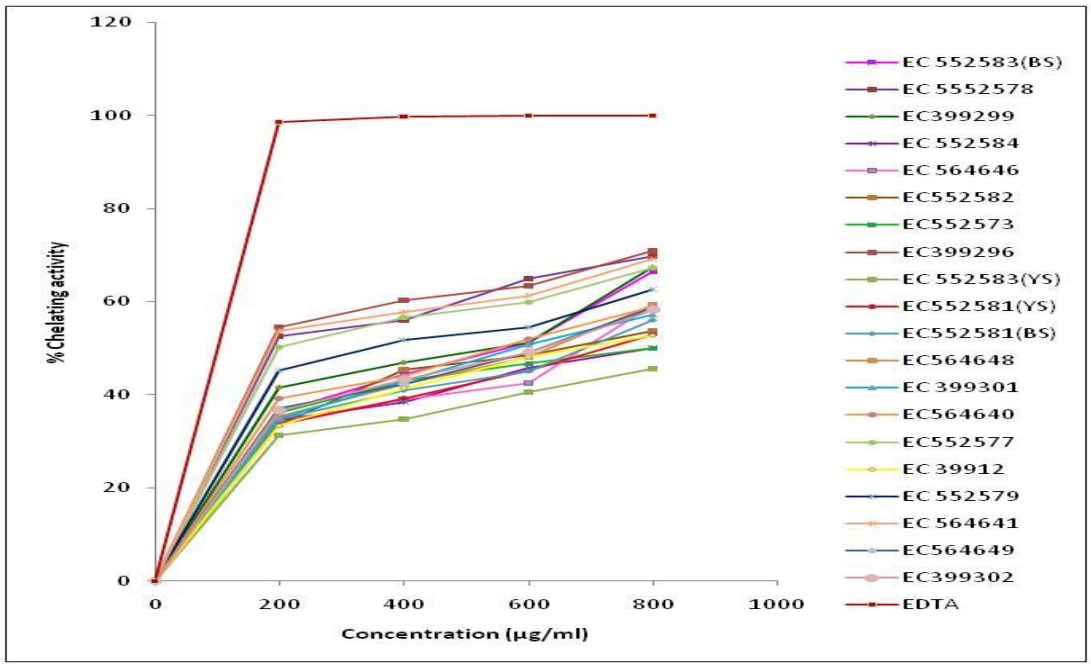

Fig.3. Superoxide radical scavenging activity in defatted meal of exotic collections of Indian mustard

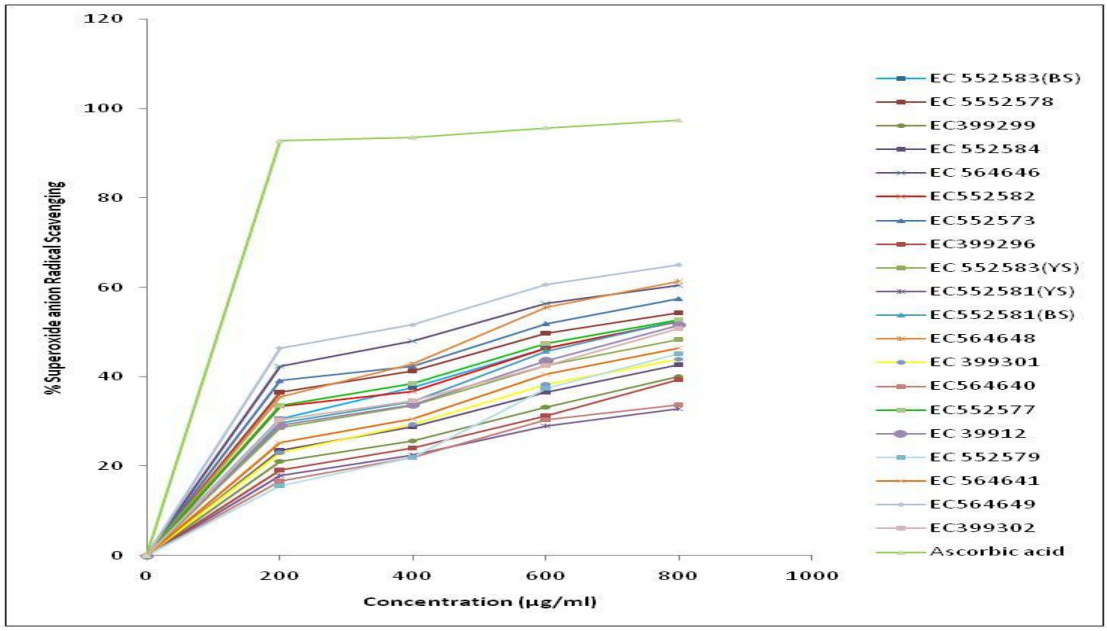

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Fig. 4. Nitric oxide activity of defatted meal in exotic collections of Indian mustard

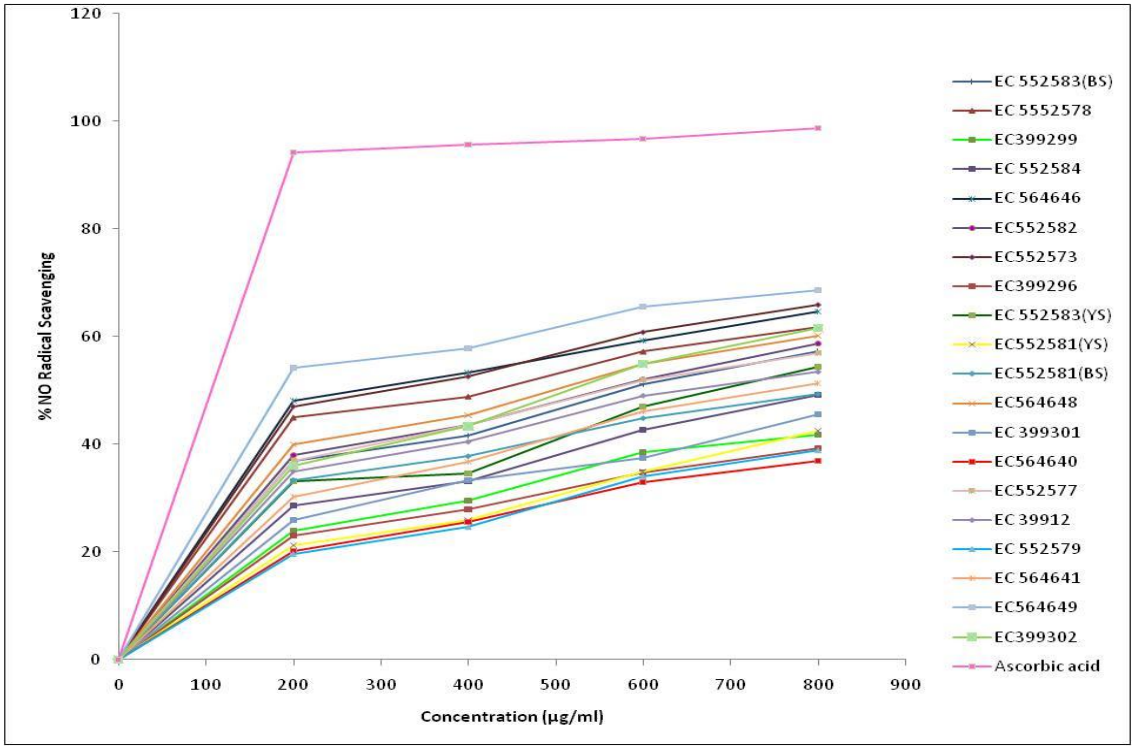

Fig. 5. Hydroxy radical activity in defatted meal of exotic collections of Indian mustard