



## Phytochemical Screening and Nutritional potentials of some High oil Yielding Exotic collections of *Brassica juncea*.

Papola Poonam<sup>1</sup>, Punetha H<sup>2</sup>, Pant Usha<sup>3</sup>, Pant A.K<sup>1</sup> and Prakash Om<sup>4\*</sup>

<sup>1</sup>Department of Chemistry, G.B.Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar 263145, INDIA

<sup>2</sup>Department of Biochemistry, College of Basic Sciences and Humanities, G.B.Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar 263145, INDIA

<sup>3</sup>Department of Genetics and Plant Breeding, College of Agriculture, G.B.Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar 263145, INDIA

<sup>4</sup>Department of Chemistry, G.B.Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar 263145, INDIA

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### Abstract

Indian mustard is a nutritionally rich oilseed crop. Present study aims to evaluate phytochemical and nutritional potentials in selected cultivars with the purpose of providing their benefits for balanced human diet consumption. 20 genotypes of *Brassica juncea* were screened for their % oil content which ranged from 36.94±2.63% to 53.33±4.67% and their phytochemical constituents that are vitamin-C, phytic acid, fibre content and tocopherol. Significant variations among these phytochemicals were observed. Vitamin C content ranged from 46.70±1.68 mg/100g to 341.10±1.01 mg/100g. Phytic acid and crude fibre percentage were found to be maximum upto 3.86±0.01% and 15.10±0.01%, whereas minimum levels were observed 2.92±0.07 % and 7.53±0.06% respectively. Maximum tocopherol content in the defatted meal of *Brassica* genotypes was observed to be 91.27±1.12 mg/100g and lowest 6.43±0.45 mg/100g. Present study might be helpful for selecting *Brassica juncea* germplasm having desirable characteristic with enhanced nutritive and health promoting qualities.

**Keywords:** *Brassica juncea*, phytate, tocopherol, nutritional potential, crude fibre.

### Introduction

“Rai” that is Indian Mustard (*Brassica juncea*) is among most common oil-seed crops grown for commercial purpose. From last few years a tremendous increase in oilseed (*Brassica* sp) cultivation can be seen<sup>1</sup>. It ranks third in whole world<sup>2</sup>. On account of oil production it comes after soyabean and palm oil and supplies nearly 7% of the world’s edible oil. In Asian countries like India and China, the brown mustard is being used for oil production which includes breeding varieties having low glucosinolates and low erucic acid content<sup>3</sup>. India amongst all the Asian countries stands first individually in acreage as well as production of rapeseed and mustard<sup>4</sup>. In India Oil obtained from mustard is used as one of the chief cooking oils and it is also accredited with therapeutic significance. It can also be used as alternative for Diesel fuels<sup>5,6</sup>. Defatted mustard cake is used as fertilizer and animal feed<sup>7</sup>. *Brassica* meal is highly nutritious, protein efficiency ratio is 2.64 which is higher on comparing with soyabean<sup>8,9</sup>.

Vegetable oil contains mixture of beneficial monounsaturated and polyunsaturated fatty acid which are helpful in decreasing cholesterol level of blood thus prevents heart from many chronic diseases. Besides uncountable medical benefits from mustard, it is also an important nutritionally favourable crop having constituent characteristics of a balanced diet. Recently *Brassica* or Cruciferous vegetables that are rich in secondary metabolites are owing increased popularity for consumption day

by day because of their nutritional aspects. Phenolic compounds are major antioxidants of Brassicaceae and beneficial compounds for human health because of their antioxidant potential. *Brassica juncea* is a rich source of nutritionally important chemical constituents such as phytate, fibre, minerals, tocopherol, vitamins and phenolic compounds<sup>10</sup>. These naturally occurring compounds present in oil seeds of mustard are characterised with strong antioxidant properties. They act as scavengers of harmful reactive oxygen species (ROS)<sup>11</sup>. Phytate are strong chelating agent. They bind metal ions and thus making them unavailable for metabolism. Vegetables are fairly good sources of dietary fiber<sup>12</sup>. Mustard has limitation for usage as food product in monogastric animals due to undigestible fiber, Present research on *Brassica* germplasm signifies the need to quantify nutritional potentials of *Brassica juncea* for crop improvement.

### Material and Methods

**Source of plant material:** The seed samples of 20 *Brassica* genotypes used in present study were obtained from the Crop Research Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India.

**Estimation of Oil Content:** Oil content was calculated by solvent extraction method using Soxhlet apparatus using petroleum ether as solvent<sup>13</sup>. The oil was dried to a constant weight and oil % was calculated using the following formula:

$$\text{Oil content (\%)} = \frac{\text{Weight of oil}}{\text{Weight of the seed}} \times 100$$

**Preparation of Defatted mustard flour:** 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 as a non-polar solvent. 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 different phytochemical analysis.

**Preparation of methanolic extract:** 0.1 g of defatted meal was mixed thoroughly with 2.0 ml of 80% methanolic solution. Methanolic extract centrifuged at 3000 rpm for duration of 10 min was used for determination of total phenol, orthodihydroxy phenol, flavonoid, phytic acid and ascorbic acid content.

**Estimation of Phytic Acid Content:** Ferric ions make complex with phytate, this is the main principle behind this test. Complex formed cannot react with thiocyanate ion to provide distinctive pink color on absorbance of 465 nm in layer of amyl. Extinction of pink color in the layer of amyl has inverse relation with the phytate ion concentration in the test sample. Method developed by Davies and Reid<sup>14</sup> and used for estimation of phytic acid by converting into ferric phytate.

**Estimation of Ascorbic acid:** Vitamin C or ascorbic acid was determined by the method developed by Law *et al.*<sup>15</sup>. 0.1g of defatted meal was homogenized with 10% trichloroacetic acid. After this centrifugation was done for 15 minutes at 5000 rpm. Extract was taken and mixed with phosphate buffer (150Mm, pH 7.4). It was incubated for sometime followed by addition of 10% trichloroacetic acid, 4% dipyrindyl solution and were added mixed properly 3% ferric chloride solution was added to it. After vortexing and incubation of 60 minutes, absorbance at 465 nm was recorded.

**Estimation of Crude Fibres:** Cellulose was taken as standard for determining Crude fibre content using the method described by Ahuja *et al.*<sup>16</sup>. To 0.1 g of seed meal 5 ml of solution B (sulphuric acid 1.25%) was mixed and set aside in water bath at 100°C for 30 minutes. After cooling well residue was washed with deionised water, on adding solution C (NaOH 1.25%) mixture was again kept in water bath for half an hour. After warming, process of cooling and washing was again repeated. Now Solution A is added to it and kept in water bath for half an hour. On cooling of mixture 50 ml of water was added into it and absorbance was recorded at the wavelength of 590 nm.

**Determination of Tocopherols:** Tocopherols were estimated by the method practiced by Kayden *et al.*<sup>17</sup>. 0.6 ml of aliquot was taken and 0.3 ml of deionised water was added into it. Following this xylene was added and properly mixed. After centrifugation for 5 minutes upper layer was carefully taken in test tube already containing freshly prepared bathophenanthroline

reagent. FeCl<sub>3</sub> was added into it and after addition of orthophosphoric acid absorbance was recorded immediately. This is Emmerie-Engel reaction, tocopherols does reduction of Fe<sup>+3</sup> ions to Fe<sup>+2</sup> ions. Fe<sup>+2</sup> ions make a colored complex which was recorded at 536 nm within 30 seconds.

**Statistical analysis:** All data of selected genotypes of *Brassica juncea* were recorded as mean ± standard deviation for three independent samples (n=3). Data were significant at p<0.05. SPSS-16 software was used for statistical analysis.

## Results and Discussion

*Brassica juncea* is a major Rabi crop and vital resource of edible oil. It is second major growing rotational crop after rice. The major use of mustard is in oil production hence content of oil in seeds of mustard is one of the most important quantitative trait in this oilseed crop. Environmental factor and genotypic character are mainly responsible for variations in its phenotypic characteristics. *Brassica* genotypes were studied quantitatively for their oil % and phytochemical assay in terms of their phytic acid, vitamin C, crude fibres and tocopherol.

The oil content varied in all the screened genotypes. EC 399296 has lowest oil content (36.94±2.63%) while EC 564640 has highest oil content (53.33±4.67%) figure-1.

Phytate combines with various essential elements such as phosphorus, calcium, iron, and zinc which perform some essential duties inside the human body as well as in plants. Due to complex formation, these essential elements are not absorbed by the body thus there is reduction in bioavailability of these elements. Phytic acid is the potent resource of phosphorus in canola meals<sup>18</sup>. Mustard Meal contains about 11.3 to 14.3 phosphorus per gram of meal<sup>19</sup>. It was found to be highest in EC 5525732 (3.86±0.01 %) and lowest in EC399299 (0.92±0.07 %). The results are shown in figure-2.

Vitamin C is a widely used food additive, to prevent food from oxidation. Oxidative and reductive properties of vitamin C forms the base of test performed for its estimation<sup>20</sup>. Vitamin C content was observed maximum in EC 399301(341.10±1.01 mg/100g) followed by EC 399302 (307.34±3.11 mg/g) while the content was found to be lowest in EC 552579 (46.70±1.68 mg/100g). The vitamin C content varied significantly among the 20 *Brassica* genotypes screened are shown in figure-3. Vitamin C content was found to be 68.3 mg per 100g for Canola<sup>21</sup>.

Reducing fibre content in seeds is one of the several approaches that have been adopted by breeders in last few years to improve the quality of meal as fibre is negatively correlated with with oil yield and protein content in the seeds<sup>22</sup>. There is variation in crude fibre content which was found lowest in EC 552581(YS) (7.53±0.06%) followed by EC552584 (7.76±0.06%) and EC 399296 (8.10±0.03%). Maximum crude fibre content found in EC 399301 (15.10±0.01%) figure-4.

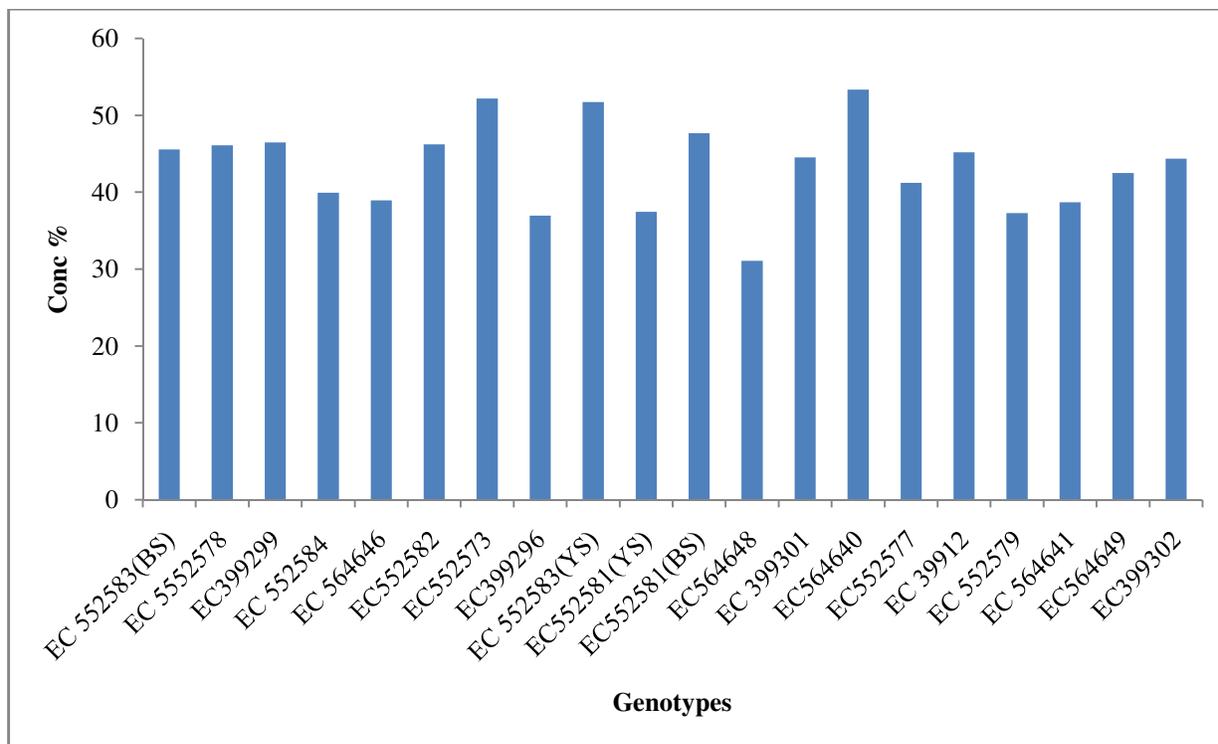


Figure-1

Percentage oil content of selected germplasm of *Brassica juncea*. Each value is represented as the mean  $\pm$  SD of triplicate measurements

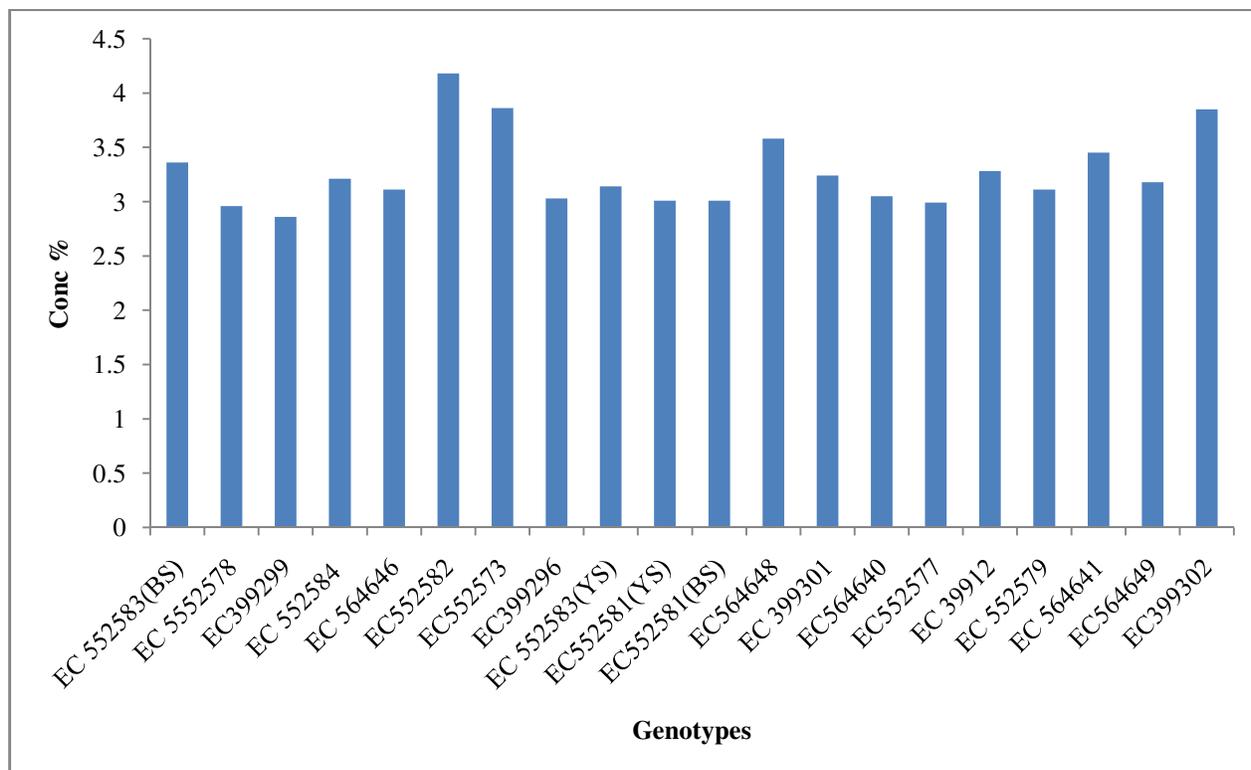
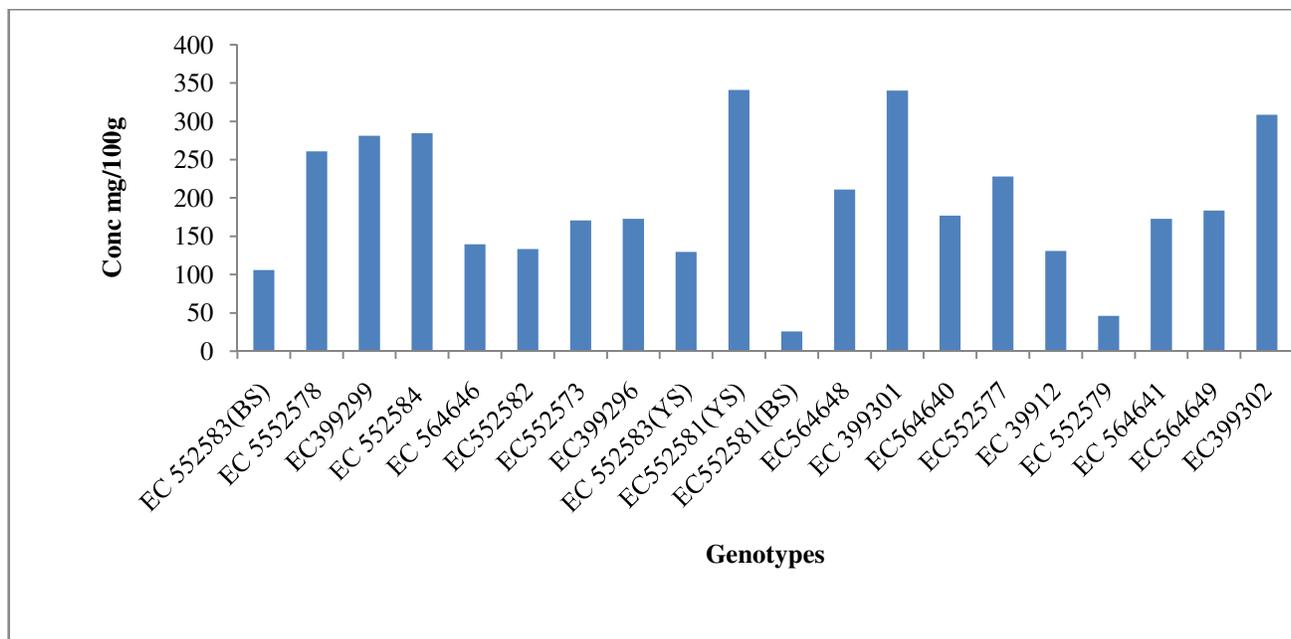
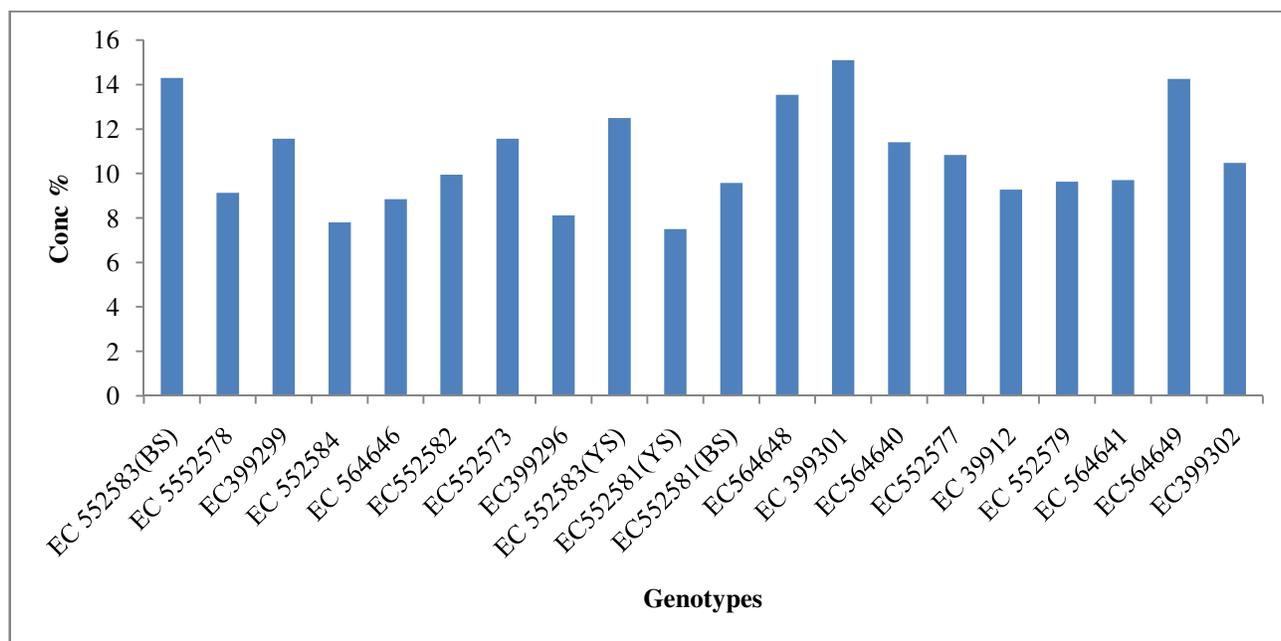


Figure-2

Phytic acid content in the methanolic extracts of *Brassica juncea* genotypes. Each value is represented as the mean  $\pm$  SD of triplicate measurements



**Figure-3**  
 Vitamin C content in the methanolic extracts of *Brassica* genotypes. Each value is represented as the mean  $\pm$  SD of triplicate measurements



**Figure-4**  
 Crude fibre percentage in the methanolic extracts of *Brassica* genotypes. Each value is represented as The mean  $\pm$  SD of triplicate measurements

Tocopherols are powerful antioxidants that have shown important role in signalling and gene expression. Among all other tocopherols,  $\alpha$ -tocopherol is the most potent form<sup>23</sup>. Antioxidant activity of tocopherol plays an significant function in the oxidative stability of the oil<sup>24</sup>. Maximum tocopherol content was observed in the defatted meal of *Brassica* genotype

EC 552581(BS) ( $91.27 \pm 1.12$  mg/100g) and lowest in EC 552582 ( $6.43 \pm 0.45$  mg/100g). Calculation was done using the linear equation ( $y=0.005x$ ,  $r^2=0.991$ ). Figure-5 is showing genotypes of Exotic Collection having different concentrations tocopherol content in them. Rapeseed oil contains 64% gamma tocopherol and 35% alpha tocopherol<sup>25</sup>.

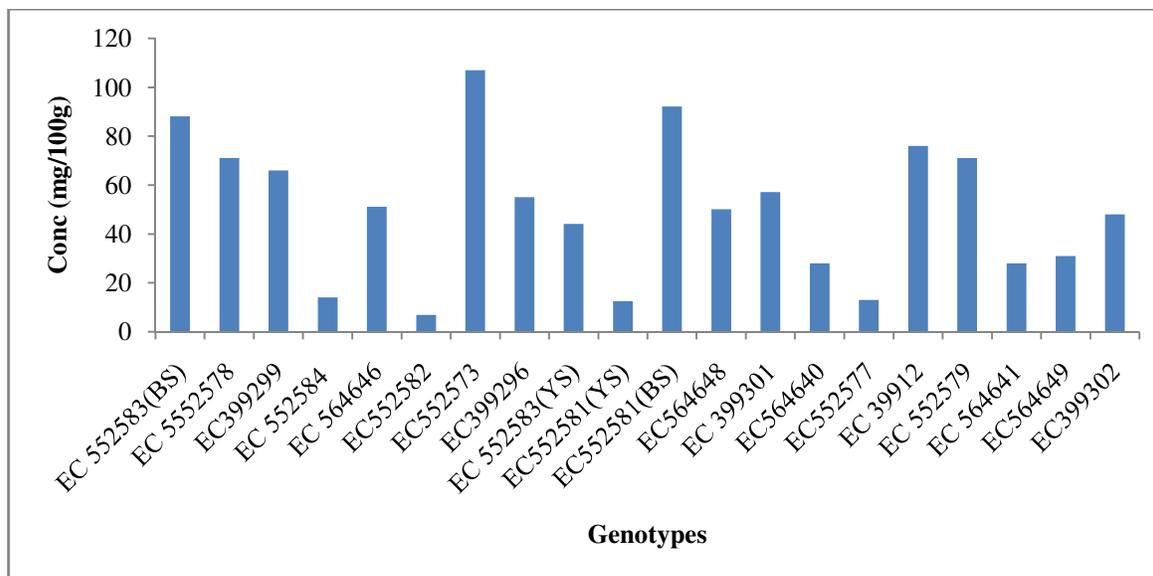


Figure-5

Tocopherol content in the methanolic extracts of *Brassica* genotypes. Each value is represented as the mean  $\pm$  SD of triplicate measurements

## Conclusion

The present study concludes that, significant variations among *Brassica juncea* cultivars was recorded in oil percentage, phytic content, ascorbic content, fibre content and tocopherol content were observed. Our study can be applied as improvement programme on crop for developing rapeseed cultivars with increased health promoting potential components as mustard has consumed worldwide consumption.

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## References

1. Trivedi N. and Dubey A., Efficient callus Regeneration and Multiple shoot induction in *Brassica juncea* var, Pusa Jaikisan, *Res. J. Recent Sci.*, **3**, 16-19 (2014)
2. Choudhary K. and Kulkarni V.S., Yeledhalli R.A. and Patil C., Study on the impact of various factors affecting the prices of mustard and its derivatives in domestic markets of India, *Int. Res. J. Agr. Econ. Stat.*, **2**(2), 154-157 (2011)
3. Merah O., Genetic Variability in Glucosinolates in Seed of *Brassica juncea*: Interest in Mustard Condiment, *J. Chem.*, **6** (2015)
4. Malan R., Walia A., Saini V. and Gupta S., Comparison of different extracts leaf of *Brassica juncea* Linn on wound healing activity, *Eur. J. Exp. Biol.*, **1**(2), 33-40 (2011)
5. Raja S.A., Smart D.S.R. and Lee R.C.L., Biodiesel production from *Jatropha* oil and its characterization, *Res. J. chem. sci.*, **1**(1), 81-87 (2011)
6. Venkateswara R.P., Compression ratio effect on Diesel Engine working with Biodiesel (JOME)-Diesel blend as fuel, *Res. J. chem. sci.*, **5**(7), 48-51 (2015)
7. Ahmad P., Sarwat M., Bhat N.A., Wani M.R. and Kazi A.G., Lam-Son Phan Tran L.S. and Zhang J., Alleviation of Cadmium Toxicity in *Brassica juncea* L. (Czern. and Coss.) by Calcium Application Involves Various Physiological and Biochemical Strategies, *Plos One*, **10**(1), (2015)
8. Delisle J., Amiot J., Goulet G., Simard C., Brisson G.J. and Jones J.D., Nutritive value of protein fractions extracted from soybean, rapeseed and wheat flours in the rat, *Plant. Foods Hum. Nutr.*, (Formerly *Qualitas Plantarum*). **34**(4), 243-251 (1984)
9. Khattab R.Y. and Arntfield S.D., Functional properties of raw and processed canola meal, *LWT - Food sci. technol.*, **42**(6), 1119-1124 (2009)
10. Crozier A., Jaganath I B. and Clifford M.N., Dietary Phenolics: Chemistry, bioavailability and effect on health, *Nat. Prod. Rep.*, **26**, 1001-1043 (2009)
11. Teets A.S. and Were L.M., Inhibition of lipid oxidation in refrigerated and frozen salted raw minced chicken breasts with electron beam irradiated almond skin powder, *Meat Sci.*, **80**, 1326-1332 (2008)
12. Motegaonkar M.B. and Salunke S.D., The Ash and Iron

- Content of Common Vegetable Grown in Latur District, India *Res. J. Recent Sci.*, **1(4)**, 60-63 (2012)
13. Official Methods of the Analytical Chemists, AOAC, Association of the Official Analytical Chemists, Washington, DC. 17<sup>th</sup> edition, (1997)
  14. Davies N.T. and Reid H., An evaluation of phytate, zinc, copper, iron and availability from soy based textured vegetable protein meat substitutes or meat extruders, *Br. J. Nutr.*, **41**, 579-595 (1979)
  15. Law W.S., Kuban P., Zha J.H., Li S.F.Y. and Hauser P.C., Determination of vitamin C and preservatives in beverages by conventional capillary electrophoresis and microchip electrophoresis with capacitively coupled contactless conductivity detection, *Electrophoresis*, **26**, 4648-4655 (2005)
  16. Ahuja K.L. and Bajaj K.L., Colorimetric determination of crude fibre in cruciferous oilseeds, *Cruciferae Newsletter.*, **21**, 61-62 (1999)
  17. Kayden H.J., Chow C.K. and Bjornson L.K., Spectrophotometric method for determination of tocopherol in red blood cells, *J. Lipid Res.*, **14**, 533-540 (1973)
  18. Nacz M., Amarowicz R., Sullivan A. and Shahidi F., Current research developments on polyphenolics of rapeseed/canola: A review, *Food Chemistry.*, **62**, 489-502 (1998)
  19. Thompson L.U., Phytate in canola/rapeseed, *In: Shahidi, F. ed. Canola and Rapeseed: Production, Chemistry, Nutrition and Processing Technology*, Van Nostrand Reinhold, New York., 173-192 (1990)
  20. Jagota S.K. and Dani H.M., A new colorimetric technique for the estimation of vitamin C using Folin phenol reagent, *Anal Biochem.*, **127(1)**, 178-82 (1982)
  21. Acikgoz E.F. and Deveci M., Comparative analysis of vitamin C, crude protein, elemental nitrogen and mineral content of canola greens (*Brassica napus* L.) and kale (*Brassica oleracea* var. *acephala*), *Afr. J. Biotechnol.*, **10(83)**, 19385-19391 (2011)
  22. Font R.B., Guta M., Del Río J.M., Fernández-Martínez and A De Haro., Seed quality analysis of Ethiopian mustard (*Brassica carinata* A. Braun) by near infrared spectroscopy, *GCIRC Bull.*, **19**, 52-56 (2003)
  23. Kumar D., Yusuf M.A., Singh P., Sardar M. and Sarin N.B, Modulation of antioxidant machinery in  $\alpha$ -tocopherol-enriched transgenic *Brassica juncea* plants tolerant to abiotic stress conditions, *Protoplasma*, **250(5)**, 1079-1089 (2013)
  24. Diedhiou D., Faye M., Vilarem G., Mar-Diop C., Sock O. and Rigal L., Physical characteristics, Chemical composition and Distribution of constituents of the Neem seeds (*Azadirachta indica* A. Juss) collected in Senegal, *Res. J. chem. sci.*, **5(7)**, 52-58 (2015)
  25. Goffman F.D. and Becker H.C., Phänotypische Variabilität des Gehalts und Musters der Tocopherole in den Samen von Winterraps (*Brassica napus*.L), *Vorträge Pflanzenzüchtung.*, **42**, 105 (1998)