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Scrutinizing Efficacy of Ethyl Methane Sulphonate on Antioxidants of Sunflower

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Abstract: In current research the scrutiny of antioxidant potential was carried out. The sunflower seeds provide a good source of antioxidant potential. Different treatments of mutagenic substance, namely EMS, was given to the SF seeds and antioxidant potential along with other biochemical components was inquired. The total antioxidant's activity was increased at 0.1%. The reducing power of seeds was elevated after the treatments of 0.5% and 0.2% as compared to the controls, where as the total phenolic and flavonoids contents were decreased after the treatments of 0.2% and 0.3% respectively. The total reducing sugar and total protein contents were boosted after the treatment at 0.3% and 0.1% respectively, where as total sugars were reduced.

Keywords: EMS: Ethyl Methane Sulphonate, F.C.R: Folin Ciocalteu's Reagent, SF: Sunflower

1. INTRODUCTION

Mutation breeding dates back to ages ago and is still enormously used to date. Mutations may happen spontaneously or they may be induced. Mutations can be introduced by using radioactive or chemical mutagens. Out of chemical mutagens, chemical EMS (ethyl methane sulphonate) is known to create large number of point mutations (Minocha *et al.,* 1962) (Hajra. 1979). In Pakistan, conventional breeding in crop improvement is considered to be less worthy than mutation breeding (Allard, 1960). Sunflower *(Helianthus annuus, L)* is an important oilseed crop worldwide and the production of sunflower as a suitable oil crop cannot be over - emphasized. The fresh green plant can be fed as silage or fodder to livestock. The seed can be eaten raw or roasted contains 36 to 45 % oil depending on variety and can be used in salads, cooking, margarine, lubricant, paint varnishes and soap production. 100 g of seed contains 20.78 g protein, 51.46 g total lipid, 3.02 grams ash, 20 grams carbohydrate and 8.6 grams fiber having overall vitality of 2445 kilojoules. It is reported that few elements including selenium can lower menace of few sorts of oncogenesis. Different minerals are present in the seeds of sunflower (USDA 2008). Oxidative damage in human body can initiate a hefty amount of diseases such as auto-immune diseases, inflammation, cardiovascular- neurological diseases, oncogenesis and aging (Zhang 2006) (Wang *et al.,* 2006). The onset of oxidative damage in cells can be protected by taking proper amount of natural antioxidants (Ozsoy *et al.,* 2008). Polyphenols of sunflower offer a great antioxidative prospective, that can be useful mutually from a technologically plus for life functional opinion (Maier, *et al.,* 2009)

2. MATERIALS AND METHODS

2.1 Biochemical Analysis

For biochemical analysis the sample was prepared in triplicates by weighing seeds and fifty percent ethanol was mixed, the ratio of sample towards solvent was 1:5 by mass per volume. Because of versatility and miscibility of ethanol with water and most of the organic solvents it is used to prepare samples. Ethanol's solvent polarity effects qualitatively as well as quantitatively on the antioxidants' of plant extracts. It is known to give huge quantity of polyphenols from plant extracts. The sample and solvent were mixed on magnetic stirrer for three hours. The liquid extract was collected after the determination of weight and the accumulation of solvent, through purification with Whatman No. 1 mesh paper. The mixing and filtration process was recurred for three times until the complete extraction was achieved. Sample was separated and removed solvent by distillation at 78 °C. The samples were kept at 4°C.

2.2 Total Protein Contents

Total protein content was checked by using the scheme reported by Lowry *et al.,* (1951). According to the reported scheme 0.5ml of test solution was taken and 2.5ml alkaline copper reagent, shaken thoroughly and kept at room temperature for 10 minutes, then 0.25ml of diluted Folin Ciocalteu's reagent (1:1v/v with water) was added. Mixture after 30minutes absorbance was taken against the blank at 750nm on Spectro-UV-Vis Double PC spectrophotometer, Labo Med, USA 11DV-60Hz or 220V-50Hz Serion Number 001151. Blank contained all the reagents and water instead of sample. The concentration of total proteins was

calculated from the standard graph that was prepared in same manner as test sample by using different concentration of the albumin.

2.3 Total Sugar Contents

The content of total sugar was estimated by the method of Montgomery (Lowry *et al.,* 1951). The sample 0.5ml was mixed with 2.5 concentrated sulphuric acid and 0.05ml 80% phenol in test tube. After that reaction mixture was mixed and kept at room temperature for 15 minutes. Finally reading was taken against the blank at 485nmon Spectro-UV-Vis Double PC spectrophotometer, LaboMed, USA 11DV-60Hz or 220V-50Hz Serion Number 001151. Blank contained distilled water instead of sample solution. The concentration of total sugar was calculated from the standard graph that was prepared in same manner as test sample by using different concentration of the glucose.

2.4 Reducible Sugars

Reducible sugars were tested by the technique reported by Montgomery (1960) (Miller 1959) According to this method, 2.0ml (0.2ml sample + 1.8d.H_2O) of test solution was mixed with 2.0ml of dinitrosalicylic acid in test tube. Heated the mixture for five minutes in boiling water bath. The tubes were cooled in tap water and color intensity was read against blank at 540nmon Spectro-UV-Vis Double PC spectrophotometer, LaboMed, USA 11DV-60Hz or 220V-50Hz Serion Number 001151. Instead of sample distilled water was used for the preparation of blank. The concentration of reducing sugar was calculated from the standard graph that was prepared in same manner as test sample by using different concentration of the glucose.

2.5 Phenolic Compounds

Phenolics were checked by FCR method of (Singleton *et al.,* 965) in which galic acid was used as standard phenolic compound. Calibration curve was made using 0.1-millilitreof 0.0 microgram/milliliter, 500microgram/milliliter, 1000 microgram/milliliter, 1500 microgram /milliliter and 2000 microgram/ milliliter solutions of gallic acid in ethanol were added to 7.9 milliliter of water, 0.5 milliliter of Folin-Ciocalteu reagent and 1.5milliliter of 20 percent Na Co3. The sample 0.1milliliter was mixed with 37.9milliliter distilled H2O, 0.5 ml FCR and 1.5 ml of 20% sodium carbonate. Distilled H2O was used as blank. The absorption was noted after two hours at 20°C at 765 nanometer on Spectro-UV-Vis Double PC spectrophotometer, LaboMed, USA 11DV-60Hz or 220V-50Hz Serion Number 001151.

Absorbance = 00.001x + 0.0203
R^2= 0.9973

2.6 Flavonoids

Flavonoids were made to check by aluminium chloride colorimetric assay (Zhishen *et al.,* 1999). The sample 1.0ml was added in 20.3mlof 5% $NaNO_2$. Post 5minutes, 0.3ml of 10 percent $AlCl_3$ was mixed. After 6 minutes, 2milliliterof sodium hydroxide were mixed after that final sample size was made using distilled H_2O to10 ml. Mixed it thoroughly and read the absorbance against blank at 510 nanometer on Spectro-UV-Vis Double PC spectrophotometer, LaboMed, USA 11DV-60Hz or 220V-50Hz Serion Number 001151. The concentrations of flavonoid compounds were calculated from the standard graph that was prepared in same manner as test sample by using different concentration of the quercetin.

Absorbance = 0.0004 (Quercetin) + 0.0389
R^2= 0.9986

2.7 Reducing Power

The reducing power of ethanol abstracts of plants were checked as by using scheme of (Oyaizu 1986) Took 0.5ml sample solution,102.5ml phosphate buffer (6.6pH) and 2.5milliliter1 percent potassium ferricyanide. The reaction mixture was kept in water bath at temperature of 50°C for twenty minutes. After that 2.5ml of 10percent T.C. A. was mixed and centrifuged at1000g for 10 minutes. Then2.5 milliliter supernatant was added in 2.5ml distilled water and 0.5milliliter of 0.1% of ferric chloride. Absorbance was read at 700nm on Spectro-UV-Vis Double PC spectrophotometer, LaboMed, USA 11DV-60Hz or 220V-50Hz Serion Number 001151. Absorbance of the sample is directly propertional to the sample's reducing power. If the absorbance will be greater, so will be reducing power.

2.7 Total Antioxidants Activity

Total antioxidant's potential was checked by modifying phospho molybdate scheme as reported by (Prieto *et al.,* 1999) in which alpha tocopherol works as a standard. A potion of 0.4 milliliter of plant extracts was mixed with 4ml of reagent (0.6M sulphuric acid, 28mM sodium phosphate and 4mM of ammonium molybdate). Covered all tubes then kept in boiling water bath at 95˚ C for 90minutes. Cooled the samples at RT and read absorbance at 695nm on Spectro-UV-Vis Double PC spectrophotometer, LaboMed, USA 11DV-60Hz or 220V-50Hz Serion Number 001151 in contrast to blank made in similar way by substituting sample with 0.1milliliter of methanol. Antioxidants activity was articulated as mg/ml counterparts of α- tocopherol. The standard error was calculated by using Microsoft excel.

Absorbance = 0.4544 (alpha tocopherol) + 0.0131

3. RESULTS AND DISCUSSION

3.1. Biochemical Analysis: The results showed that the total sugars of sunflower seeds reduced with increasing concentration of EMS treatment. As compared to the control all the treatment concentrations reduced the total sugars content. Highest total sugar was observed in control seeds where as the lowest one was observed in the seeds treated with 0.5% EMS. (Anil *et al.,* 2012) reported increased reducing sugar in mulberry plant treated with 0.3 % EMS. Our findings were similar and showed that EMS treatment had escalating effect on the reducing sugars.

The reducing sugars were boosted at the treatment of 0.3% EMS, which was much more than the control seeds. But increase in the concentration has detrimental effect. The increase in protein contents are similar to the findings of (Anil *et al.,* 2012) where EMS induced the production of protein contents in mulberry plant treated with 0.3%. The outcome of treatment in present study had showed not so much noticeable effect on the total protein contents of the sunflower seeds. The total protein contents were slightly enhanced at the treatment of 0.5% EMS which was bit higher than controls. It showed that EMS with 0.1% concentration can be used to elevate the protein contents of SF seeds. The lowest levels were noticed in seeds treated with 0.5% EMS.

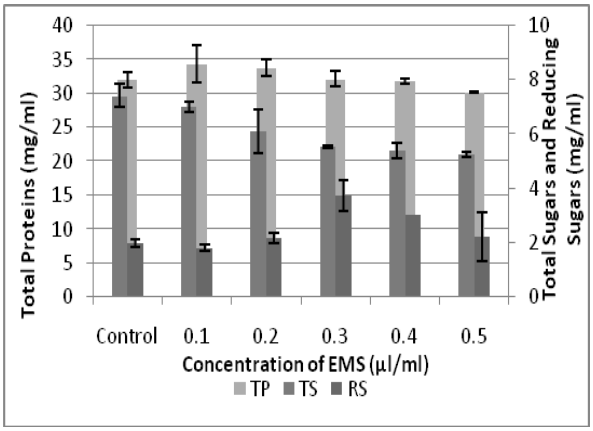

Fig. 1: Effects of different concentrations of EMS on total proteins, total sugars and reducing sugars in seeds of sunflower.

3.2. T**otal Phenolics:** The Total Phenolics compounds are well known chain breaking antioxidants (Anil *et al.,* 2012). Phenolic compounds are highly indispensible constituents of plants. Their capability to scavenge radicals is due to the presence of hydroxyl groups (Shahidi and Wanansundara. 1999). There is also antioxidative aspect of phenolics compounds (Hatano *et al.,* 1989). It is reported that polyphenolics compounds hampers onset of mutation and cancer in humans, if about 0.1g every day taken from a diet packed with branches and vegies (Duh *et al.,* 1999) Radical scavenging activity is associated with phenolics contents of plants. Due to the elevated redox potential, polyphenolics work as reducing agents, donate hydrogen and capture oxygen singlets .EMS probably induce variations in the amount of phenolics constituents and affect antioxidant's activity. The total phenolics of SF seeds did not show any elevated effect after given EMS treatment. These were higher in controls but moved to declined levels after treatment. In graphs it can easily be witnessed that increase in EMS was almost inversely proportional to phenolics. The least quantity of total phenolics was observed to be found in the seeds treated with 0.2% EMS and highest were observed in controls.

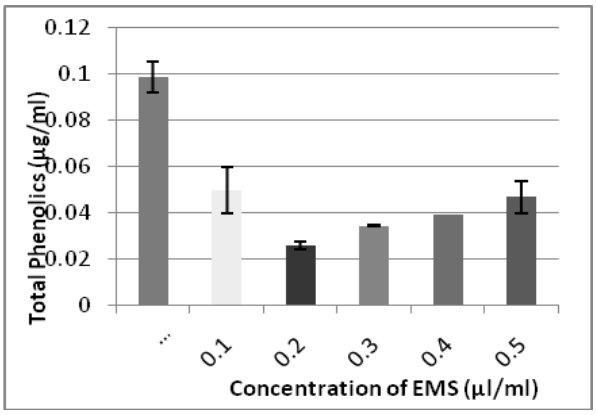

Fig: 2: Effects of different concentrations of EMS on total phenolics (µg/ml) in seeds of sunflower.

3. 3. The Total Flavonoids: Natural phenolics materials are prominent EMS (0.1%) was found to increase flavonoid contents of mulberry plant. The results of current study showed that EMS did not have any apparent change in the contents of flavonoids of the sunflower plant. Whereas the highest amount of flavonoids was found to be in controls and lowest in 0.3% of EMS.

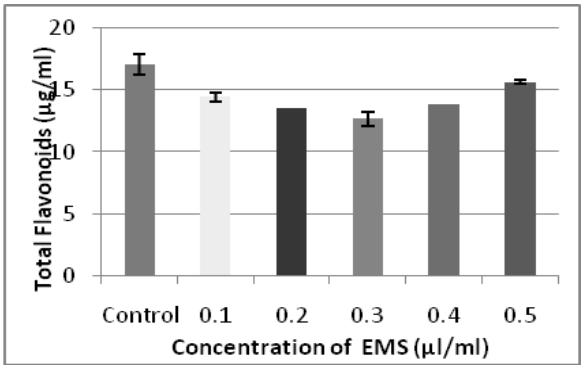

Fig: 3: Effects of different concentrations of EMS on total flavonoids (µg/ml) in seeds of sunflower.

3. 4. **Reducing power:** The presence of reductants causes reduction of the Fe3+ - ferricynide complex to the ferrous form. Therefore, Fe2+ can be checked by the formation of Perl's Prussian Blue at 700nm.The reducing power of SF seeds was boosted with elevated concentrations of EMS. The samples made from all treated seeds posses' greater activities contrasting control. The highest reducing power was noticed in the seeds treated with 0.5% and 0.2% of EMS, which were higher than controls. It showed that EMS can be helpful in boosting up reducing power.

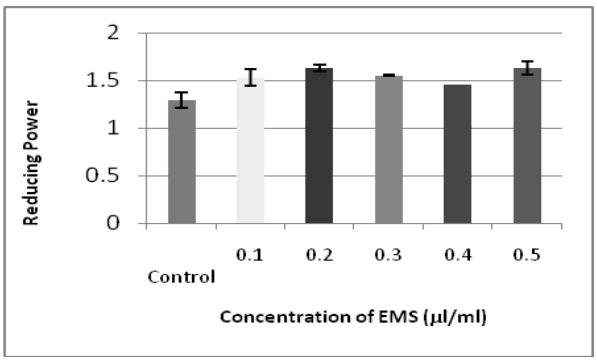

Fig: 4: Effects of different concentrations of EMS on reducing power of seeds of sunflower.

3. 5. **Total Antioxidant's activity:** There are number of ways to determine antioxidant activities. The results obtained are usually scattered because of chemical intricacy of extracts, usually fabrication of dozen compounds with diversity of functional groups, polarity and chemical behavior and the type of test employed. EMS probably might induce variations quantitatively in the profiles of phenolics constituents and hence affect antioxidant's activity. In present study the antioxidants activity was measured by applying a little bit difference to the phosphomolybdate method which was formulated by Prieto *et al.,* (1999) usingα-tocopherol as a standard. The total antioxidant's activity of sunflower seeds was observed after treating seeds with EMS. The highest antioxidant's activity was observed at 0.1% which was much more as compared to the control.

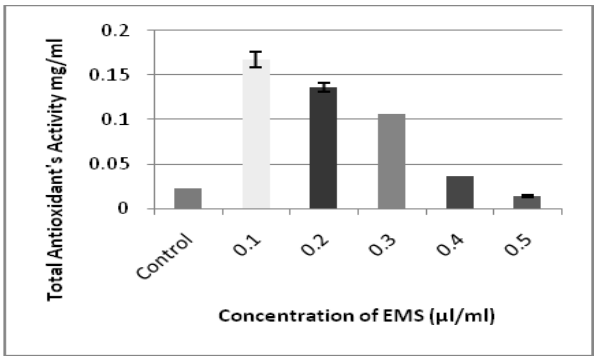

Fig: 5: Effects of different concentrations of EMS on antioxidant's activity (mg/ml) of seeds of sunflower.

6. CONCLUSION

The findings of current study are in consistence with the data presented formerly on Sunflower. After mutation induction there was apparent change in plant's nutritional components.

The biochemical components like total sugars, reducing sugars and protein contents showed different elevated and decreased levels. The total sugars were decreased with the consequent increase in EMS concentrations whereas the reducing sugars were increased at 0.3% EMS treatment. The outcome of present study treatments showed slightly increased levels of protein contents. The slightly enhanced protein contents were found to be in 0.1% EMS treatment.

The total phenolics contents did not show any elevated effect after treatment. Total phenolics compounds were higher in control plant but gradually moved to declivity in response to treatment. The least amount of phenolics was observed in 0.2% EMS. The Flavonoids contents did not show any noticeable change after given treatments. Highest amount of flavonoids was found controls than in treated seeds. The reducing power of SF seeds elevated after every treatment. Reducing power was found to be higher than controls. Highest reducing power was noticed in 0.5% and 0.2%. The total antioxidant's activity also increased after treatment of 0.1% EMS.

It can be concluded from the above findings that certain concentrations of EMS might alter the naturally occurring biochemical components and antioxidants. Further research on current findings needs to be done to get further improved results.

REFERENCES:

Allard, R. W. (1960) Principles of Plant breeding. Wiley, New York; 139–142.

Anil K., H. V., and T. S. Muralidhar (2012) Sourav Acharya, Manas Jyoti Das, Munirajappa. EMS Induced Morphometric Biomass and Phytochemical Variations in Morus Species (Genotype RFS135). American Journal of Experimental Agriculture; 3(1): 43-55, 2013

Cakir A., A. Mavi A. Yıldırım, M. E. Duru M. Harmandar and C. Kazaz (2003) Isolation and characterization of antioxidant phenolics compounds from the aerial parts of Hypericum hyssopifolium L. by activity-guided fractionation. Journal of Ethno pharmacology; 87: 73–83.

Duh P. D., Y. Y. Tu, and G. C. Yen (1999) Antioxidant activity of water extract of harn jyur (Chyrsanthemum morifolium Ramat). Lebensmittel- Wissenschaft und-Technologie-Food Science and Technology; 32: 269–277.

Hajra. N. G. (1979) Induction of mutations by Chemical Mutagens in tall indica rice. Indian Agric; 23: 67–72.

Hatano T, R. Edamatsu. A Mori (1989) Effect of interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. Chemical and Pharmaceutical Bulletin; 37: 2016–2021.

Kregel K. C., and H. J. Zhang (2006). An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am J Physiol 292: 18-36.

Lowry OH, N. Rosenbrough AL, Farr and RJ. Randall (1951) Protein measurement with the Folin Phenol Reagent. J Biol Chem; 193: 265-275.

Lide, D. R., (2000). Handbook of Chemistry and Physics 81st edition. CRC press. ISBN 0-8493-0481-4

Maier T, A. Schieber, D. R Kammerer and R. Carl (2009) Residues of grape (Vitis vinifera L) seed oil production as a valuable source of phenolic antioxidants. Food Chem; 112: 551-559.

Minocha J. L., and T. J. Arnason (1962) Arnason Mutagenic effectiveness of ethyl methane sulfonate in barley. Nature; 196: 499.

Montgomery R. (1960) Further studies of the phenol sulphuric acidreagent for carbohydrate. Biochem Biophysc Acta; 448: 591-576.

Miller G. L. (1959) Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Anal Chem; 31(3): 426-428.

Oyaizu M. (1986) Studies on products of browning reactions: antioxidative activities of browning reaction prepared from glucosamine. Japanese Journal of Nutrition; 44: 307-315.

Ozsoy N, A, Can, R, Yanardag, and N. Akev (2008) Antioxidant activity of Smilax excelsa leaf extracts. Food Chem; 110: 571-583.

Prieto P., M. Pineda, and M. Anguilar (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E. Anal Biochem; 269: 337-341.

Singleton V. L, and J. A Rossi. (965) Colorimetry of total phenolics with phospho-molybdic-phosphotungstic acid reagents. Am J EnolVitic 1; 16: 144-158

Shahidi F. and PKJPD. Wanansundara. (1999) Phenolic Antioxidants. Critical Reviews in Food Science and Nutrition 32: 67-103.

Tanaka M., C. W. Kuei Y. Nagashima and T. Taguch (1998) Application of antioxidative maillrad reaction products from histidine and glucose to sardine products. Nippon Suisan Gakkaishil; 54Pp.

USDA (2008). Accessed on, 2009/07/25, http://www.nal.usda.gov/fnic/foodcomp/

Windholz, M. (1976). The Merck index: an encyclopedia of chemicals and drugs (9th ed.). Rahway, N.J., U.S.A: Merck. ISBN 0-911910-26-3

Wang J. S., and M. A. Maldonado (2006). The ubiquitin-proteasome system and its role in inflammatory and autoimmune diseases. Cell Molecular Immunol 3: 255-261.

Zhishen J., T. Mengcheng and W. Jianming (1999). The determination of Flavonoids content in mulberry and their scavenging effects on superoxide radicals. Food Chem; 64: 555-559.