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Research Article

Antioxidant-based Evaluation of Seed Extracts of Two Maize Varieties for Medicinal Purpose

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Abstract: The aim of this study was to explore the medicinal value of seeds of two maize varieties obtained from Cereal Crop Research Institute, Pirsabak, Nowshera, Pakistan. The antioxidant system was evaluated in the methanolic seed extract using DDPH, ABTS, Phosphomolybdate and hydrogen peroxide scavenging activities. Here, we showed that both Azam and Iqbal varieties exhibited high scavenging activities at minimum and maximum extract concentrations against free radicals at a level comparable to ascorbic acid (standard). DPPH was the only free radical that was scavenged 50% less in comparison to standard whereas for ABTS, hydrogen peroxide and phosphomolybdate both varieties respond more or less the same as for ascorbic acid. Moreover, compared to Azam, Iqbal variety showed better antioxidant mechanism at 0.75 mg mL^{-1} and 1.5 mg mL^{-1} extract concentration against ABTS and phosphomolybdate. These high scavenging activities of the methanolic extract indicate that seeds of both varieties possess good antioxidant system in removing of free radicals and thus can be a potent medicinal plant for the treatment of oxidative stress and can be considered as good health supplement.

Keywords: Antioxidant, maize seeds, medicinal

1. INTRODUCTION

Reactive oxygen species (ROS) are the chemical species that are produced in the cell due to metabolic imbalance. These include the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (HO•) [1]. Under optimal condition, the formations of these species are at very low level in plants that can be increased upon various stresses. The effects of various environmental stresses such as drought, salinity, chilling, metal toxicity, UV-B radiation, and pathogen attack can directly increase ROS production [2]. These ROS when reaches the toxic level can damage the DNA, proteins and lipids [3]. Plants have a well established antioxidant defense system to minimize the toxic level of ROS [4]. To counteract with the toxic effect of free radicals that are produced in the body, different natural products are also used [5]. Plants and its extracts are considered as a good source for various novel therapeutical products. According to World Health Organization (WHO) 60-80 % populations of the developed countries are also using medicinal plants as a source of medicine to cure different diseases [6]. Various reports are available that highlights the importance of crude extract and natural products from medicinal plants against oxidative stress [7, 8]. Most of the vegetables and their different parts (roots, leaves, oil seeds and fruits) possess natural antioxidants capacity [9]. In addition, some plants have vital medicinal values and can be used against cancer because of the presence of anticancer substances [10, 11, 12, 13]. Plants are good source for natural antioxidant compounds; therefore they help to protect the cell from oxidative stress damages [14]. Also, due to the presence of different compounds such as hydroxyl group, phenolic compound and conjugated ring structures, plants have the antioxidant ability by scavenging

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these free radicals through hydrogenation [15]. Different studies have confirmed a relation between consumption of diets such as vegetables, red wine and fruits with a decrease in degenerative diseases [16]. The present study was conducted on seed crude extract of different maize varieties with the possible aim to find out the medicinal value by using natural antioxidant mechanism in scavenging of free radicals.

2. MATERIAL AND METHODS

2.1. Collection of Samples

Seeds of two maize varieties (Iqbal and Azam) were collected from Cereal Crop Research Institute (CCRI) Pirsabak, KPK, Pakistan for different antioxidant assays in 2015.

2.2. Preparation of Extract

Seeds were first shade dried and then powdered with blinder. 15 mg powder of each variety was dissolved in 100 mL of methanol in a beaker and placed for eight days. Each sample was shaked properly. Afterwards, the filtration of the extract was performed with Whatman paper and placed at room temperature until all methanol was evaporated. The solid extract was collected in eppendorf tubes for further analyses.

The stock solution of each crude extract as well as ascorbic acid (reference) was prepared in methanol with a concentration of 3 mg mL^{-1} and was then further diluted to 1.5, 0.75 and 0.37 mg mL^{-1}.

2.3. DPPH Free Radical Scavenging Assay

0.2 mL of seed extract was taken from stock (3 mg mL^{-1}) and also from each dilution (1.5, 0.75 and 0.37 mg mL^{-1}) and 0.8 mL of DPPH solution was added to each test tube. The tubes were properly shaked and kept in the dark at room temperature for 30 minutes. The absorbance was then measured at 517 nm by spectrophotometer.

DPPH radicals scavenging activity (%) =

(AC-AS/AC) ×100

Where, AC = Absorbance of the control solution,

AS = Absorbance of the sample

2.4. Scavenging Assay for ABTS Free Radical

This assay was performed according to the procedure of Re et al., (1999) with some modifications [17]. Briefly, 7 mM ABTS free radical solution was added to potassium persulfate (2.45 mM) solution and kept overnight in the dark. A solution of dark colour was obtained indicating the presence of ABTS cation radical. The ABTS solution was diluted with 50 % methanol before use. The diluted solution was then placed for 20 minutes at room temperature in dark. About 0.2 mL of the sample and 0.8 mL of the ABTS solution was mixed in the cuvette for one minute and the reduction in absorbance was measured. The final absorbance was noted after two minutes. The inhibition was measured using the following formula,

ABTS scavenging activity (%) = [(CA-SA)/ (CA)] × 100

2.5. Phosphomolybdate Free Radical Scavenging Assay

Phosphomolybdate scavenging assay was performed according to Umamaheswari and Chatterjee [7]. About 0.1 mL was mixed with 1 mL of reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a test tube. All test tubes were covered with aluminium foil and kept at 95ºC in water bath for 90 minutes followed by cooling at room temperature. The absorbance was then measured at 765 nm by spectrophotometer. By using the following formula, antioxidant capacity was measured:

Phosphomolybdate scavenging activity (%) = [(CA-SA) / (CA)] ×100

2.6. Hydrogen Peroxide Scavenging Assay

This assay was performed as described by Ruch et al. (1989). 2 mM solution of H_2O_2 was prepared in 50 mM phosphate buffer (pH 7.4). Each sample extract (0.1 mL) was transferred to test tubes and the volume was increased to 0.4 mL by addition of 50 mM phosphate buffer (pH 7.4). Then 0.6 mL solution of H_2O_2 was added to all test tubes and vortexed properly. The solution was kept for 10 minutes and the absorbance was measured at 566 nm by spectrophotometer. The methanol was used

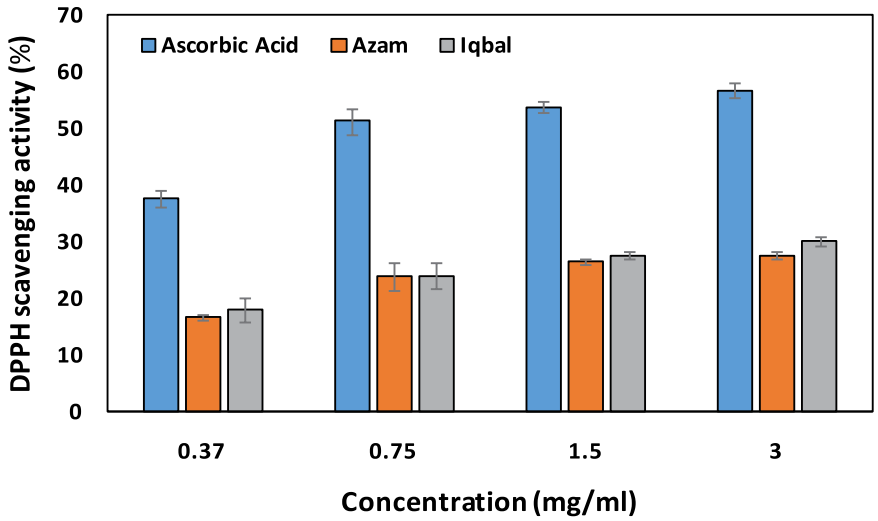

Fig. 1. Comparison of ascorbic acid and methanolic seed extract of maize varieties on the scavenging of DPPH free radical.

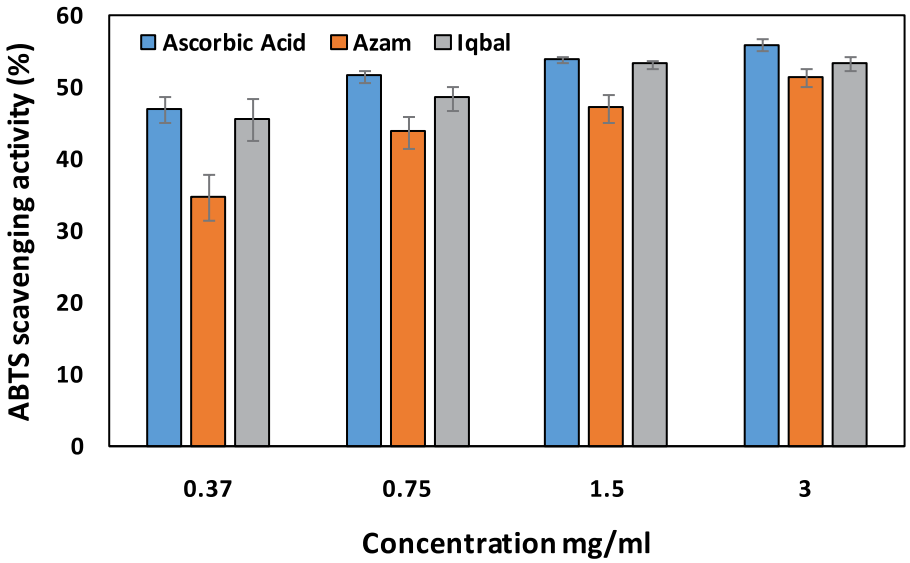

Fig. 2. Comparison of ascorbic acid and methanolic seed extract of maize varieties on the scavenging of ABTS free radical.

as a reference and the ascorbic acid as a standard. The activity is expressed as % hydroxyl radical scavenging [18]. The ability of the samples to scavenge hydrogen peroxide was measured by the following formula:

Hydrogen peroxide scavenging activity = (1- SA/CA) × 100.

2.7. Statistical Analyses

Statistical analysis was performed using unpaired t-test. SigmaStat 12.0 was used for checking the constant variance and normal distribution of data. Moreover, the Mann-Whitney rank sum test was used to analyze samples that did not follow normal Gaussian distribution.

3. RESULTS

3.1. DPPH Free Radical Scavenging Activity

DPPH is a stable free radical and is widely used to evaluate the scavenging ability of natural compounds of plants. The antioxidant activity

of a substance can be considered as its ability to detoxify DPPH free radical. Compared to ascorbic acid (standard), the scavenging ability of seed extract of both varieties (Azam and Iqbal) was more than 50% lower. Moreover, we found an increase in the scavenging of free radical with an increase in the concentration of the extract (0.37 to 3 mg mL^{-1}) indicating that the seed extracts have enough antioxidant system that can reduce the stable DPPH free radical (Fig. 1).

3.2. ABTS Free Radical Scavenging Activity

Due to observed increase in the scavenging of DPPH free radical, ABTS was also analysed which is used for reflecting the antioxidant ability in a complex mixture of various plants. As expected, the seed extract of both varieties showed good antioxidant capacity. At a concentration of 0.37 mg mL^{-1}, the seed extract of Azam and Iqbal scavenged about 30 and 45 % of the free radicals, respectively followed by 47 and 50 % detoxification of ABTS free radical

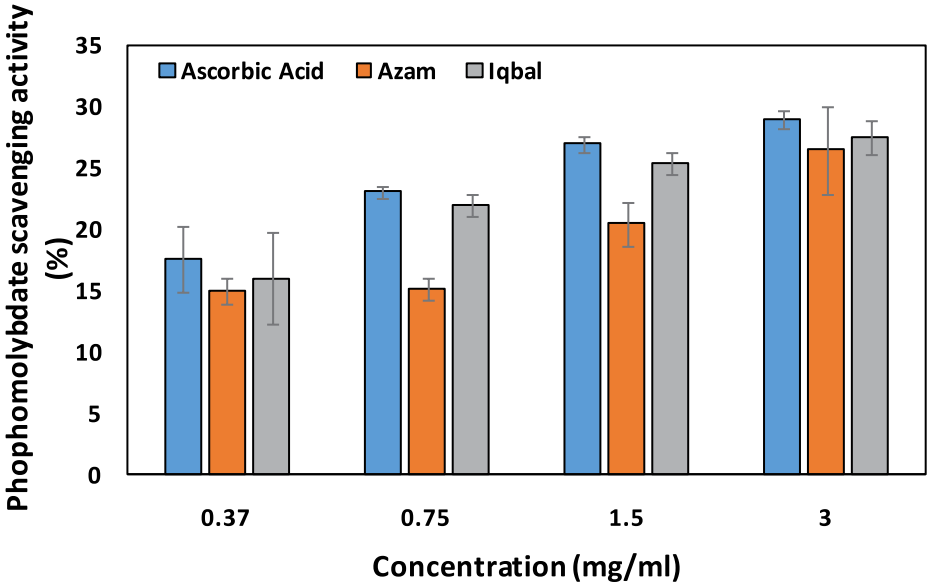

Fig. 3. Comparison of ascorbic acid and methanolic seed extract of maize varieties on the scavenging of phosphomolybdate free radical.

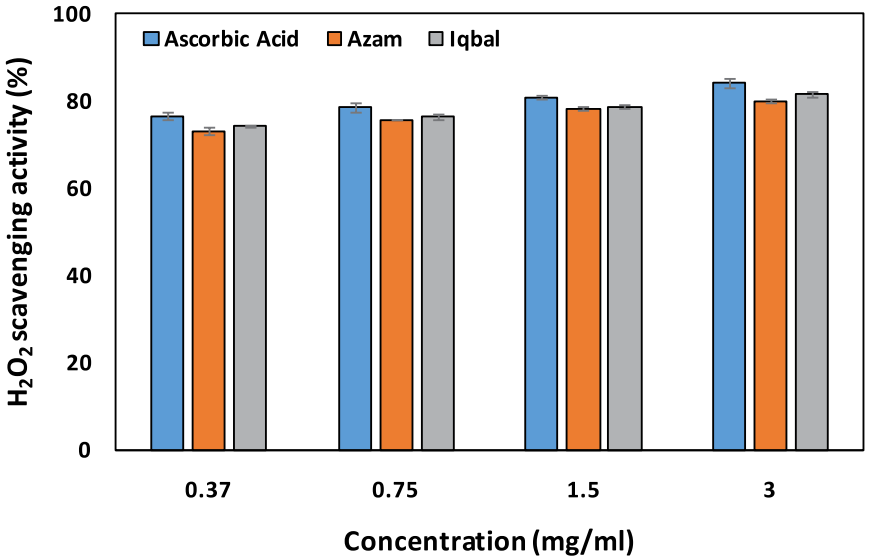

Fig. 4. Comparison of ascorbic acid and methanolic seed extract of maize varieties on the scavenging of H_2O_2 free radical.

at a concentration of 3 mg mL^{-1} (Fig. 2). The result obtained clearly indicates that the seed extract of maize possess potent ABTS scavenging activity at a level comparable to the ascorbic acid.

3.3. Phosphomolybdate Free Radical Scavenging Activity

The data obtained here showed that at low concentration (0.37 mg mL^{-1}) both the varieties exhibited about 15 % antioxidant activities. This percentage was high with an increase in the extract concentration and almost close to the level as observed for the standard. This result suggests that methanolic seed extract of both varieties at minimum and maximum concentration have potent scavenging properties in minimizing the detrimental effect of free radical when compared with the ascorbic acid.

3.4. Hydrogen Peroxide Free Radical Scavenging Activity

H_2O_2 is the most stable ROS that is produced in plants under various stress conditions. This ROS can be detoxified by a well established antioxidant mechanism present in different cellular compartments. Our results showed that the H_2O_2 were efficiently reduced by the methanolic extract of seed of both varieties. Initially, 75 % of the H_2O_2 were scavenged at a concentration of 0.37 mg mL^{-1}. This detoxification efficiency was even more and almost comparable with the ascorbic acid when higher concentration (3 mg mL^{-1}) of the extract was used (Fig. 4). Taking together, this reflects that the seed extract of Azam and Iqbal exhibited efficient antioxidant capacity in coping with the toxicity of ROS.

4. DISCUSSION

The presence of natural antioxidants in plants is vital in inhibition or prevention of the deleterious effect of free radicals that are responsible for oxidative stress. The evaluation of these natural antioxidant activity from plant sources can be best studied by the extensively used of free radicals such as DPPH, ABTS, phosphomolybdate and hydrogen peroxide. In present study, the scavenging ability of methanolic seed extract of two maize varieties was performed with the aim to investigate its medicinal value. Here, we reported that both Azam and Iqbal varieties exhibited strong antioxidant mechanism against free radicals such as ABTS, DPPH, hydrogen peroxide and phosphomolybdate as compared to standard. ABTS, Hydrogen peroxide and Phosphomolybdate showed a scavenging activity which was closed to ascorbic acid whereas DPPH had almost 50 % less scavenging activity with that of standard. All these observations suggest that the methanolic extract of both the varieties of maize showed a stronger ABTS, hydrogen peroxide and phosphomolbydate antioxidant activity as compared to DPPH. These results are nearly similar with Hogerman et al. [19], who also reported that the plants rich in medicinal values will have the ability to scavenge free radicals and can be used as health supplement [20]. Extracts obtained from raw seeds registered higher DPPH radical-scavenging activity than both the dry- and wet-heat treated seed samples. The study also showed that the higher the concentration of extracts, the higher the capacity to scavenge free radicals [21]. Hydrogen peroxide itself is not very active, but it can sometimes be toxic to cells, since it may give rise to hydroxyl radicals inside the cell [22]. Similar study with methanol extract of roots also exhibited higher DPPH, ABTS, hydrogen peroxide and phosphomolybdate radical -scavenging activity for investigating medicinal importance of the plant [23].

5. CONCLUSIONS

The seeds of the tested maize varieties (cv. Azam and cv. Iqbal) have the capability to cope with ROS and, thus, can be used as a good source of medicinal value against various oxidative stress-related diseases and ultimately for maintaining good health.

6. REFERENCES

1. Shinde, A., J. Ganu & P. Naik. Effect of free radicals & antioxidants on oxidative stress. *Journal of Dental & Allied Sciences* 1: 63–66 (2012).
2. Elstner, E.F. Mechanisms of oxygen activation in different compartments of plant cells. In: *Active Oxygen, Oxidative Stress and Plant Metabolism*. Pell, E.J. & K.L. Steffen, (Ed.). American Society of

Plant Physiologists, Rockville, MD, USA, p. 13–25 (1991).
3. Sivanandham, V. Free radicals in health and diseases. *Pharmacology Online* 11: 1062-1077 (2011).
4. Pang, C.H. & B.S. Wang. Oxidative stress and salt tolerance in plants. *Progress in Botany* 69: 231–245 (2008).
5. Kokate, C.K. & A.P. Purohit. *Text Book of Pharmacognosy* 29: 317-18 (2004).
6. Shirwaikar, A., R. Verma, R. Lobo & A. Shirwaikar. Phytotherapy Safety aspects. *Natural Product Radiance* 8(1): 55-63 (2009).
7. Umamaheswari, M. & T.K. Chatterjee. In vitro antioxidant activities of the fractions of *Coccinnia grandis* L. leaf extract. *African Journal of Traditional, Complementary and Alternative Medicines* 5(1): 61-73 (2008).
8. Kil, H.Y., E.S. Seong, B. K. Ghimire, I.M. Chung, S.S. Kwon, E.J. Goh, K. Hoe, M. J. Kim, J.D. Lim, D. Lee & C.Y. Yu. Antioxidant and antimicrobial activities of crude sorghum extract. *Food Chemistry* 115: 1234-1239 (2009).
9. Rababah, T.M., N.S. Hettlarachchy & R. Horex. Total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola and ginkgo extracts, vitamin E and tert. butylhydroquinone. *Journal of Agricultural and Food Chemistry* 52: 5183–86 (2004).
10. Crabbe, P. Some aspects of steroid research based on natural product from plant orgin. *Bulletin des Societes Chimques Belges* 88: 5-7 (1979).
11. Mitscher, L.A., S. Drake, S.R. Gollapudi & S.K. Okwute. A modern look at folkloric use of anti-infective agents. *Journal of Natural Products* 50: 1025-40 (1987).
12. Cook, N.C. & S. Saman. Flavonoids: Chemistry, metabolism, cardioprotective effects and dietary sources. *Nutritional Biochemistry* 7: 66-76 (1996).
13. Marino, M., Bersani C, & Comi G. Impedance measurements to study the antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. *International Journal of Food Microbiology* 67: 18795 (2001).
14. Kahkonen, M.P., A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kujala & M. Heinonen. Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry* 47: 3954-3962 (1999).
15. Shahidi, F. & J.P.K.P.D. Wanasusdara. Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition* 32: 67–103 (1992).
16. Ruch, R..J., S.J. Cheng & J.E. Klaunig. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis* 10: 1003-1008 (1989).
17. Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang & C. Rice-Evans. Antioxidant activity applying an improved ABTS radical cation decolorisation assay. *Free Radical Biology and Medicine* 26: 1231-1237 (1999).
18. Babu, B.H., B.S. Shylesh & J. Padikkala. Antioxidant and hepatoprotective effect of Alanthusicicifocus. *Fitoterapia* 72: 272-277 (2001).
19. Hogerman, A.E., K.M. Riedl, G.A. Jones, K.N. Sovik, N.T. Ritchard, P.W. Hartzfeld. High molecular weight plant polyphenolice (tannins) as biological antioxidants. *Journal of Agriculture & Food Chemistry* 46:1887-1892 (1998).
20. Huang, D., Ou B. & Prior, R.L. The chemistry behind antioxidant capacity assays. *Journal of Agriculture & Food Chemistry* 53: 1841–1856 (2005).
21. Enujiugha, V.N., Y. Justina, S.A. Talabi, Malomo, I.O. Aderonke. Dpph radical scavenging capacity of phenolic extracts from African yam bean (*Sphenostylis stenocarpa*). *Food & Nutrition Sciences* 3: 7-13 (2012).
22. Halliwell, B. *The biological toxicity of free radicals and other reactive species*. In: Free radicals and food additives; Aruoma, O.I., & B. Halliwell (Ed.). Taylor and Francis, London, UK (1991).
23. Khan, W.U., R.A. Khan, M. Ahmed, Khan, M.W. Pharmacological evaluation of methanolic extract of *Cyperus scariosus*. *Bangladesh Journal of Pharmacology* 11: 353-58 (2016).