IJPSR (2016), Vol. 7, Issue 8 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 March, 2016; received in revised form, 08 April, 2016; accepted, 31 May, 2016; published 01 July, 2016

EVALUATION OF PHYTOCHEMICALS, REDUCING POWER, ANTIOXIDANT ACTIVITY AND *IN-VITRO* LIPID PEROXIDATION ACTIVITY OF WHEAT GRASS JUICE

S. N. Jangle and P. Padmanabhan *

Department of Biochemistry, Rural Medical College, Pravara Institute of Medical Sciences (DU), Loni - 413736, Ahmednagar, Maharashtra, India.

Keywords:

Wheat Grass Juice, Phytochemicals, Free Radicals, Antioxidant, Antilipidperoxidation

Correspondence to Author:
P. Padmanabhan

Associate Professor
Department of Biochemistry,
Rural Medical College, Pravara Institute of Medical Sciences (DU),
Loni - 413736, Ahmednagar,
Maharashtra, India.

Email: preetipadmanabhan@gmail.com

ABSTRACT: Oxidative stress is a condition due to excessive production of free radicals than the ability of the antioxidant system to counteract them. Antioxidants scavenge and suppress the deleterious effects of free radicals. Both exogenous and endogenous sources of antioxidants are important in pathological conditions. Exogenous antioxidants include dietary sources and even include medicinal plants. These medicinal plants contain phytochemicals which contribute to the beneficial antioxidant activity. Wheat germinated over a period of 9-10 days is generally called wheat grass. The purpose of this work is to determine the phenolic, flavonoids, flavonols and total alkaloids from wheat grass juice. Free radical scavenging activity of wheat grass juice was evaluated by using DPPH , nitric oxide, hydroxyl radical and total antioxidant power by FRAP assay and antilipidperoxidation activity by using mice liver and brain. Wheat grass juice is found to have good antioxidant action in various models due to its polyphenols and phytochemicals.

INTRODUCTION: Oxidative stress in human beings is a condition when the sensitive homeostasis between free radicals and antioxidants is disturbed. This is due to excessive production of free radicals than the capacity of antioxidants to counteract on them [1].

The radicals are capable of damaging macromolecules and cause various human diseases such as neurodegenerative disorders, cardiovascular diseases, diabetes mellitus and different forms of cancer.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(8).3436-40

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (8).3436-40

Antioxidants scavenge and suppress the deleterious effects of free radicals. Under normal conditions the endogenous antioxidants in human body are active defense against free radicals. But, in pathological conditions, the additional antioxidants from diet as well as natural sources such as from medicinal plants are necessary for the recovery to normalcy [2].

These medicinal plants contain phytochemicals such as total phenolic compounds, flavonoids, flavonols, tannins, nitrogen compounds such as alkaloids, amines, vitamins, terpenoids which contribute to the beneficial antioxidant activity [3].

The consumption of wheat grass in the Western world started in 1930s, as a result of experiments by Charles F. Schnabel in his attempts to popularize the medicinal plant. The usage of wheat grass juice for therapeutic purpose was developed

and popularized by Dr. Ann Wigmore (1909 – 1996) U.S.A, founder- director of Hippocrates Health Institute, Boston, U.S.A. It is reported to be safe and effective for the treatment of cancer, rheumatoid arthritis, ulcers, diabetes, gastritis, fatigue, anemia, asthma, eczema, hemorrhoids, skin problems, body odour, constipation and even high blood pressure [4, 5]. Wheat (*Triticum aestivum* L.) belongs to family Gramineae. It is considered as important component of human diet, particularly in developing countries. Wheat germinated over a period of 9-10 days is generally called wheat grass. Thus, wheat grass refers to the young grass of common wheat plant. It is rich in chlorophyll, amino acids, minerals, vitamins and enzymes [6].

The aim of the present study is to evaluate the phytochemicals, reducing power, antioxidant activity and *in – vitro* lipid peroxidative capacity of wheat grass juice.

MATERIALS AND METHODS:

Preparation of wheat grass juice:

25grams of wheat grains were allowed to germinate for a period of 9 days in soil, under normal conditions. On 10th day the green leaves obtained were collected. The wheat grass juice was obtained by crushing the green leaves in 100 ml of double distilled water and subjected to double filtration in order to collect the fresh filtrate for use and remove uncrushed insoluble matter. The fresh filtrate was further diluted with distilled water so as to obtain a concentration of 250 µg/ml and the various aliquots were used for the further experiments as wheat grass juice.

Phytochemical analysis:

The wheat grass juice was subjected to preliminary phytochemical analysis of total phenolic compounds, flavonoids, flavonols by methods described earlier by the authors Padmanabhan and Jangle; 2012 [7 "a"] . The DPPH ,nitric oxide and hydroxyl radical scavenging activity The total antioxidant activity by FRAP assay, reducing property was determined, by the methods described by authors Padmanabhan and Jangle; 2012 [7 "b"]. The anti lipid peroxidation of wheat grass juice was carried out in mouse liver as well as in brain homogenate of D-galactosamine induced hepatic damage (Padmanabhan and Jangle ;2014) [8] The 10% liver homogenate was centrifuged at 3000 rpm for 10 minutes and the supernatant obtained was used. 0.5 ml of the supernatant obtained from liver homogenate was added to 0.5 ml saline in a series of test tubes to which wheat grass juice was added in concentration ranging from 50µg/ml to 250µg/ml. The mixture was incubated for 15 minutes at 37°C. 10% trichloroacetic acid was added to stop the reaction.

The tubes were centrifuged at 3000 rpm for 20 minutes and the entire supernatant obtained was treated with 0.25 ml 1% thiobarbituric acid in 0.2 N NaOH. Further, the tubes were subjected to boiling for one hour at 95˚C. Thereafter the tubes were cooled to room temperature. The absorbances were measured on spectrophotometer at 532 nm. TBARS were estimated by the method of Fraga et al (1984)[9]. The 10% brain homogenate in phosphate buffer pH 7.4 was centrifuged at 3000 rpm for 10 minutes and the supernatant was used. 0.25 ml of supernatant of brain homogenate was taken in a series of test tube to which wheat grass juice was added in a concentration range of 50µg/ml to 250µg/ml. Further 0.05 ml of 15mM ferrous ammonium sulphate was added to enhance lipid peroxidation. All the test tubes were incubated for 30 minutes at 37˚C in a water bath.1 ml of 10% trichloroacetic acid was added and centrifuged for 20 minutes at 3000rpm. The entire supernatant obtained in each test tubes were treated with 1 ml of 0.8g/dl thiobarbituric acid and kept at 100˚ C for 20 minutes. The test tubes were allowed to cooled and thereafter centrifuged and the absorbances of the supernatants were measured on spectrophotometer at 530 nm.

RESULTS:

TABLE 1: PHYTOCHEMICAL ANALYSIS

Sr.no	Phytochemical	Mean ± SD
1.	**Total phenolic compounds** µM of gallic acid equivalent /g of wheat grass	301.74 ± 13.76
2.	**Flavonoids** mg rutin equivalent /g of wheat grass	10.29 ± 4.57
3.	**Flavonols** mg rutin equivalent /g of wheat grass	0.44 ± 0.08

Values are MEAN ± SD of three measurements

TABLE 2: ANTIOXIDANT POWER BY FRAP ASSAY

Sr.No	Conc n µg/ml	Wheat Grass Juice	Standard BHT	Standard Ascorbic Acid
		mM ferric ions reduced to ferrous ions /litre FRAP reagent(MEAN ± SD)		
1.	50	21.43 ± 2.95	4.36 ± 3.68	4.18 ± 0.88
2.	100	64.96 ± 2.58	18.22 ± 4.88	44.67 ± 4.21
3.	150	107.67 ± 8.18	34.93 ± 1.69	84.25 ± 4.26
4.	200	289.14 ± 18.17	225.14 ± 11.86	238.85 ± 4.09
5.	250	365.43 ± 27.96	403.70 ± 6.38	509.26 ± 2.65

Values are MEAN ± SD of three measurements

TABLE 3: DPPH RADICAL SCAVENGING ACTIVITY

Sr.No	Conc n µg/ml	Wheat Grass Juice % Inhibition	Standard BHT % Inhibition	Standard Ascorbic Acid % Inhibition
1.	50	9.68 ± 0.38	34.33 ± 0.34	58.67 ± 1.07
2.	100	12.87 ± 0.34	45.28 ± 1.08	76.67 ± 0.60
3.	150	16.17 ± 0.22	57.00 ± 1.48	91.33 ± 1.68
4.	200	23.62 ± 1.24	64.66 ± 2.34	94.67 ± 0.25
5.	250	25.97 ± 1.41	68.89 ± 1.98	96.00 ± 0.20

Values are MEAN ± SD of three measurements

TABLE 4: HYDROXYL RADICAL SCAVENGING ACTIVITY

Sr.No	Conc n µg/ml	Wheat Grass Juice % Inhibition	Standard BHT % Inhibition	Standard Ascorbic Acid % Inhibition
1.	50	27.27 ± 15.11	77.01 ± 3.04	60.00 ± 11.55
2.	100	54.54 ± 28.15	79.31 ± 4.30	65.71 ± 9.18
3.	150	63.63 ± 9.74	81.60 ± 1.76	71.43 ± 8.73
4.	200	69.70 ± 4.63	82.76 ± 0.66	74.29 ± 10.03
5.	250	75.75 ± 9.73	93.10 ± 3.04	77.14 ± 4.37

Values are MEAN ± SD of three measurements

TABLE 5: NITRIC OXIDE SCAVENGING ACTIVITY

Sr.No	Conc n µg/ml	Wheat Grass Juice % Inhibition	Standard Potassium Nitrate % Inhibition
1.	50	11.78 ± 8.16	21.21 ± 1.01
2.	100	19.46 ± 13.85	26.60 ± 3.82
3.	150	38.04 ± 11.12	30.97 ± 2.54
4.	200	39.39 ± 8.02	39.05 ± 16.10
5.	250	39.73 ± 8.71	44.42 ± 23.62

Values are MEAN ± SD of three measurements

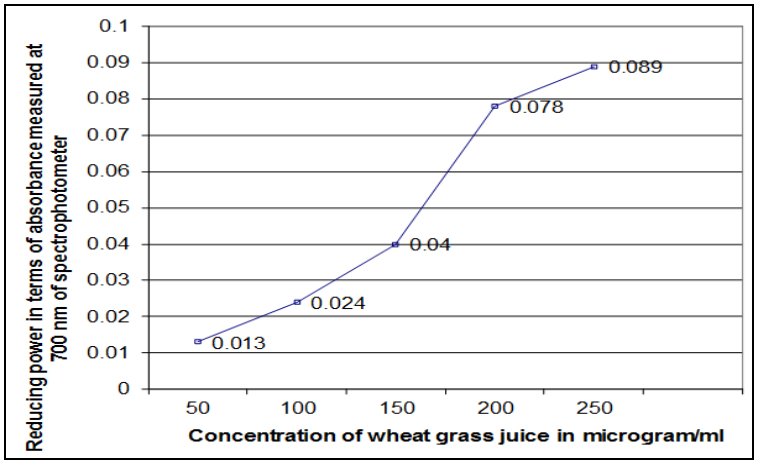

FIG. 1: REDUCING POWER OF WHEAT GRASS JUICE

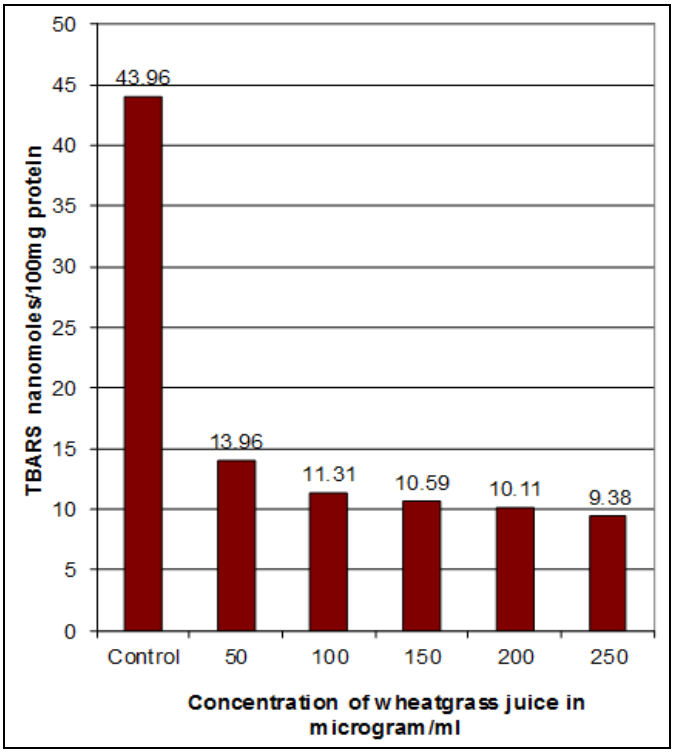

FIG. 2: *IN-VITRO* ANTILIPID PEROXIDATION OF WHEAT GRASS JUICE USING LIVER HOMOGENATE

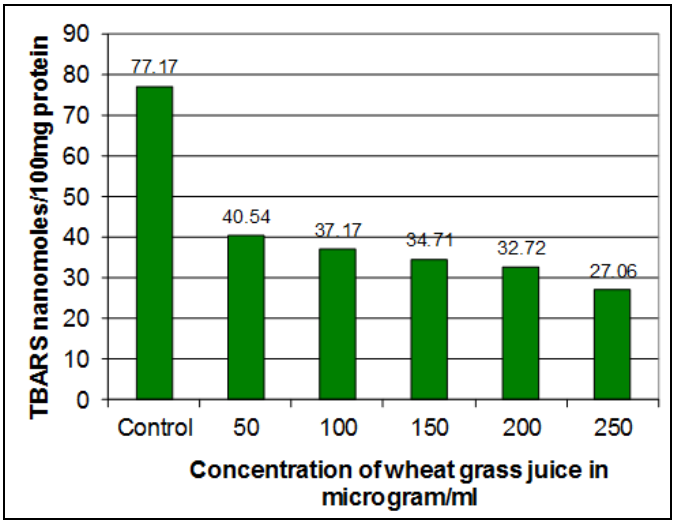

FIG.3: *IN-VITRO* ANTILIPIDPEROXIDATION OF WHEAT GRASS JUICE USING BRAIN HOMOGENATE

DISCUSSION: The total phenolic compounds are a group of various phytochemicals of prime importance that occur along with various types of flavonoids, flavonols, alkaloids, saponins etc. Various authors have reported their therapeutic properties such as antimicrobial, antioxidant and anticarcinogenic [10], anti-inflammator [7 "c"], hepatoprotective [8], antioxidant [7"a"] and antimutagenic [11]. Correlation was reported by Tawaha *et al* (2007) [12], between the phenolic compounds and antioxidant activity of selected Jordian species. Ayoola *et al* (2008) [13] reported the antioxidant activities of selected medicinal plants used in malarial therapy. The compounds, mainly responsible for the antioxidant effect are a class of phenolic compounds including flavonoids and their derivatives [14].

In the present study **Table 1** shows that, the wheat grass juice is found to be rich in total phenolic compounds, flavonoids and flavonols. In a recent study carried out by Durairaj *et al* (2014) [1], estimated the phenolic compounds and flavonoids in wheat grass. Our results are in agreement with these reports.

The total antioxidant capacity of wheat grass juice as depicted in **Table 2**, determined by FRAP assay. The antioxidant power of wheat grass juice was concentration dependent and better one when compared with standard BHT and ascorbic acid.

The ferric reducing power of wheat grass juice was dose dependent as per **Table 2**. Such observations was reported by Durairaj (2014) [1]. Our observation is in line with this. Thus FRAP assay of antioxidant activity may be referred analogously to total reducing power.

Free radical scavenging assay was carried out using DPPH denoted in **Table 3** and hydroxyl radical scavenging potential is shown in **Table 4.** DPPH scavenging action of wheat grass juice was dose dependent, but less compared to standard BHT and ascorbic acid, while hydroxyl radical scavenging action comparable to that of BHT and ascorbic acid and concentration dependent. Nitric oxide quenching action as per **Table 5** is dose dependent and compared well with standard potassium nitrate solution.

In vitro antilipid peroxidation with liver and brain of mice treated with D-galactosamine (to induce increased lipid peroxidation in vivo) was carried out by incubating liver and brain homogenate respectively with various concentrations of wheat grass juice. Concentration dependent decrease in TBARS formation was noted, indicating antilipid peroxidation capacity of wheatgrass juice as shown in **Fig. 2** and **3**. Such antilipid peroxidation of our herbal preparation HP-4 was reported by authors

Padmanabhan and Jangle (2012) [8 "b"] using cod liver oil.

Duh *et al* (1999) [15] reported that polyphenols are directly related for contribution to antioxidant action. Antioxidants are specific to the various species of reactive oxygen[16].

The various degrees of inhibition of various oxidants observed due to various scavenging models used in this study. Some of the antioxidants may directly interact with superoxide, hydroxyl radicals, singlet oxygen quencher or can act directly as reducing agent or hydrogen donor [17]. Hamberger and Hustettman (1991) [18] are of opinion that the crude extracts of plants are pharmacologically more active than their isolated active principles due to the synergistic effects of various phytochemicals like alkaloids, flavonols etc present in the whole extract.

Thus, the wheat grass juice rich in phenolic compounds, flavonoids, flavonols and alkaloids is having antioxidant activity no wonder that has large therapeutic potential in prevention and treatment of chronic diseases Singh *et al* (2012) [5].

ACKNOWLEDGEMENTS: Nil

CONFLICT OF INTEREST: Nil

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How to cite this article:
Jangle SN and Padmanabhan P: Evaluation of Phytochemicals, Reducing Power, Antioxidant Activity and *in-vitro* Lipid Peroxidation Activity of Wheat Grass Juice. Int J Pharm Sci Res 2016; 7(8): 3436-40.doi: 10.13040/IJPSR.0975-8232.7(8).3436-40.