International Conference on Innovative Research in Engineering, Management and Sciences
International Journal of Scientific Research in Computer Science, Engineering and Information Technology
© 2019 IJSRCSEIT | Volume 4 | Issue 9 | ISSN : 2456-3307

In vitro Antioxidant and Antihemolytic Activity of Triticum aestivum Linn.

Ananda Vardhan Hebbani[1*], Swathi Lakshmi P[2], Vinitha M Hebbar[2], Mythreyi S Jamadagni[3], Yokshitha Reddy Bathula[4], Asha BM[1]

[1]Department of Biotechnology, New Horizon College of Engineering, Bangalore-560103, Karnataka, India
[2]Molecular Genetics Lab, NIMHANS, Bangalore-560078, Karnataka, India
3Department of Plant Biology, University of Houston – Clear Lake, Houston, Texas 77058, USA
[4]Department of Environmental and Forest Biology, State University of New York, College of Environmental Science & Forestry, Syracuse, New York 13210, USA

Correspondence: *Dr.Ananda Vardhan.H, Associate Professor & HOD, Dept. of Biotechnology, New Horizon College of Engineering, Marathalli ORR, Bangalore – 560 103, Karnataka, India*

Phone: 09448296073, E.mail:hod_bte@newhorizonindia.edu, h.anandavardhan@gmail.com

ABSTRACT

Plant based medicines are understood and widely accepted as alternative therapies for many diseases. The present work investigated the antioxidant and antihemolytic properties of extracts of Triticum aestivum (wheatgrass) have been analyzed and quantified for the phytochemical constituent's such as total phenolics, tannins, and flavonoid contents. High–performance liquid chromatography revealed the presence of many phytoconstituents with medicinal value. Antioxidant and RSA was determined by in vitro assays such as hydrogen peroxide, nitric oxide, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging and total reductive ability. These results proved to be promising and were confirmed by its ability to mitigate free radical induced erythrocyte damage.

Keywords : Green grass, antioxidant, antihemolytic, Triticum aestivum, phytochemical, free radical damage, erythrocytes.

I. INTRODUCTION

Noxious dietary patterns and sedentary lifestyle practices (smoking, consumption of alcohol, prolonged exposures to harmful UV rays, ionizing radiations, etc.) have shown to cause pathological damage in the body's cells and tissues resulting in deleterious impacts[1]. This causes a morbid stress in the body, leading to the generation of unstable free radicals [2,3,4]. These free radicals, by the virtue of their chemical properties, tend to be highly reactive so as to pair up with other molecules, atoms or even a single electron in order to achieve the state of stability. They get involved in a variety of chemical reactions in the body such as hydrogen abstraction, termination, addition, disproportionation, etc., to reach a more "bound" state, leading to the utilization of more free molecular oxygen, causing to what is known as "oxidative" stress in the body[3].

Free radicals induced damage in the biological systems is most clearly comprehended using blood as

a promising model[5]. Blood being the fluid connective tissue of the body becomes easily susceptible to this damage due to free radical induced physico chemical damage of RBC membranes leading to hemolysis[6], which subsequently creates an "oxygen" deficit condition in the body leading to hypoxia and related chronic ailments like cancer, diabetes, neurological damages and cardiovascular diseases[4].

Traditional herbal medicines, today have been shown to act as what is called "alternate medicines", due to their less toxic and multi-targeted potential in the body[6]. They are a repository of a widespread amount of antioxidants, which can be used to the development of what is known as "novel" drugs[7]. The present work is one such attempt to look at the protective effect of wheat grass against free radical induced erythrocytic. Wheatgrass being an herbal medicine, is known to have attracted the attention of the society for the prevention of many diseases[8].

Triticum aestivum **(wheat grass)** (commonly known as bread wheat) is one of the most abundantly grown wheat specie in the world. *Triticum ae* sp. belongs to the family Poaceae[9]. The plants of this family are all monocots, primarily grasses. Some commonly found plant species belonging to this family include *Cynodon dactylon, Poa annua, Zea mays*, etc. (North American Range Plants*, James L. Stubbendieck, Stephan L. Hatch, Charles H. Butterfield*) [10]. Wheatgrass is generally grown throughout the year and is most commonly seen to propagate in almost all regions of the world. Fifteen to twenty species of wheatgrass have been documented, of which have been reported to occur in India[11].

Wheatgrass is a good pool of mineral nutrients. It is a rich repository of iron, phosphorus, magnesium, manganese, copper & zinc[13]. Wheatgrass is an excellent headspring of tocopherols with other phytochemical compounds[11]. It helps to trigger metabolic activities, maintains the alkalinity, and hence reducing the over-acidity in the blood. It also aids in reinstating the healthy cells by acting as a detoxifier[12]. They tend to possess various antioxidant potentials and thus is seen to inactivate many free radical induced damages in the human body[14]. Typically, wheatgrass is shown to be a house of - reducing sugars, anthraquinones, saponins, flavonoids, tannins, alkaloids, terpenoids, and phenolics, which help majorly in antioxidant activities[15]. It's said to have anticancer activities according to the Traditional system of medicines[9].

Wheat grass has shown to be a promising modal for curing a variety of diseases[16]. Its juice is being proved to be hypolipidemic[17] hypoglycemic [18] hepatoprotective [19] in rat models and the chemopreventive action of wheat grass [20.] Pilot studies to a considerable extent were performed on human models wherein wheat grass juice was found to reduce transfusion requirements in patients with thalassemia major [21].Also, the plant has been shown to be [22] (through a randomized,double bind,placebo-controlled study of the plant against spirulina) an effective protectant against lipid peroxidation. It also has a major role in curing active distal ulverative colitis by tests performed on controlled trials [23] . Works on human chronic myeloid leukemia cells lines revealed the antiproliferative and apoptotic activities of the plant [24] .The plant juice has to a certain extent been proven to improve the hematological conditions in patients with breast cancer [25] . It has shown to have the property of optimizing blood sugar levels and so has been examined to prove its effectiveness in curing diabetes mellitus[26]. Quantitative assays performed on the plant revealed that a particular chemical called MPa (**methylpheophorbide a**), has potent abilities in curing cancer [27]. Acute oral toxicity levels for the plant was being demonstrated, revealed a significant no mortality results in Swiss Albino mice trials with a dosage of 2000 mg/kg seen for 14 days [28.] All these results have shown that the plant has a significant pharmacological benefit and thus can be used in the long run to cure a variety of diseases.

Design of Experiments

Plant Material and extraction

The grass of ***Triticum aestivum*** used in the study was grown indoors in earthen pots filled with 2.5 inches of soil (3 parts) mixed with compost (1 part). Harvesting was done on the tenth day when the grass grows approximately 5-6 inches tall for further experimentation [29]. Ethanolic extract (10% w/v) of wheat grass powder was prepared using the method of Arpita *et al.*, 2012. wherein the freshly harvested grass was freeze dried in vacuum (2.4 X 10^{-2} mB) with a condenser temperature set at -49^{0}C for 6 h. Aqueous extracts of ***Triticum aestivum*** (AET) was prepared by using sequential extraction method [30]

Estimation of total Phenolic, Flavonoid and Tannins content

Total phenolic content [TPC] expressed as milligrams of gallic acid equivalents [GAE] per gram of dry extract [mg GAE/g of dry extract], total flavonoid content [TFC] expressed as milligrams of rutin equivalents [RE] per gram of dry extract [mg RE/g of dry extract] and the total tannin content [TC] of both the individual phytoextracts were determined using the standard methods [30]

Identification and Quantification of the Polyphenols using HPLC

Polyphenolic contents of the extract were obtained chromatographically by separating them on a reverse phase Luna 5 µm C18 (2) (100 Å, LC Column 250 x 4.6 mm). Gallic acid, tannic acid, catechin, β sitosterol, rutin, quercetin, and betain (100µg/ml of each) were used as standard polyphenols.

In vitro Antioxidant Assays:

Hydrogen peroxide (H_2O_2) scavenging ability:

H_2O_2 radical scavenging ability of the extracts was examined according to the method employed by Tuba and Gulcin [31], with few modifications. To 3.4 ml of a plant extract and the standard antioxidant - ascorbic acid (dissolved at different concentrations of 5-100 µg/ml in phosphate buffer - 50 mM, pH 7.4), 0.6 ml of H_2O_2 was added and incubated at room temperature for 10 min. The decrease in the absorbance of H_2O_2 upon oxidation was monitored at 230 nm spectrophotometrically against a suitable blank (phosphate buffer alone). BHA was used as a standard antioxidant, control (phosphate buffer and H_2O_2) was prepared and the percentage inhibition was calculated using the expression:

Percentage inhibition (%) = (OD of control – OD of extract/OD of control) × 100 (1)

The extracts concentration providing 50% of inhibition (EC_{50}) was calculated from the graph of percentage inhibition plotted against extract concentration.

Nitric oxide (NO) scavenging activity:

NO radical scavenging abilities of plant extracts were assessed by using the method employed by Royer *et al.* [32].The color intensity of the chromophore formed as a result of diazotization of nitrite with sulfanilamide and subsequent coupling with naphthyl ethylenediamine hydrochloride was measured colorimetrically at 546 nm against a suitable blank (2 ml of H_2O and 0.6 ml Griess reagent). Control (200 µL SNP, 800 µL H_2O and 300 µL Griess reagent) was run and the percentage inhibition was calculated using equation 1 mentioned above and compared with ascorbic acid which is used as a standard antioxidant. EC_{50} values for the plant extracts were estimated.

DPPH free radical scavenging activity

Antioxidant activities of the plant extracts were assessed based on DPPH free radical scavenging activity using Blois method [33] with a few changes. Ascorbic acid prepared at similar concentration ranges like the aqueous plant extracts was used as a standard positive control. Percentage inhibition was

calculated by comparing tests with control (3 ml of DPPH) using equation 1.

Preparation of erythrocyte suspension

Blood samples from healthy male/female (non-smoker and non-alcoholic) volunteers were collected into heparinized vaccuettes through venipuncture after taking informed consent. After a gentle swirling, the tubes were centrifuged at 1500 g for 10 min at 4°C and the plasma and buffy coat were removed. The resulting erythrocytes were washed thrice with 10 volumes of PBS (PBS - 10 mM having NaCl - 150 mM, NaH_2PO_4 - 1.9 mM and NaH_2PO_4 - 8.1 mM, pH 7.4) and centrifuged again at 1500 g for 5 min. The thick fluffy coat was removed with care after each centrifugation. Erythrocyte suspension stock of 10% v/v was prepared in PBS and kept at 4°C and used within 6 h.

In vitro hemolysis assay

To look at the protective antioxidant effect of the aqueous plant extract on the free radical induced oxidative damage on human erythrocytes, *in vitro* hemolysis assay was performed as adapted by Girish *et al.* [34].

II. RESULTS

Phytochemical Evaluation of the Aqueous Extract of *Triticum aestivum*

The concentrations of the polyphenols vary depending on many environmental factors and the extraction techniques employed. Aqueous extract in the present study also was found to be very rich in different polyphenolic components, with the details being presented in Figure 1.

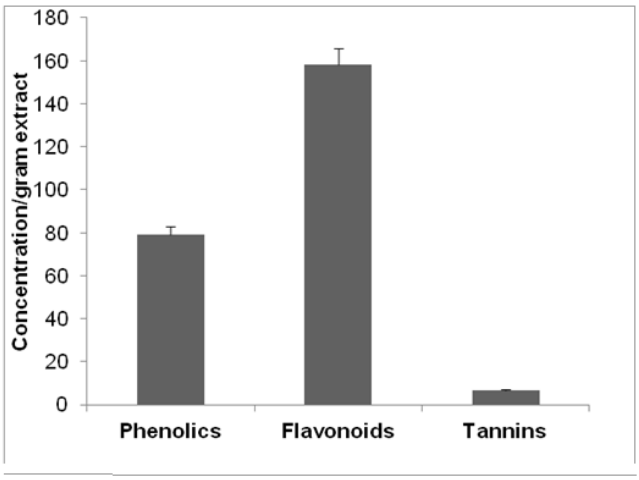

Fig. 1: Evaluations of phenolic, flavonoids and tannin profiles of the plant extract of wheatgrass.

Polyphenol quantification using HPLC

The individual polyphenols present in the plant extract was characterized using HPLC with columns specific for separating the hydrophobic compounds and UV detection at 280 nm as seen in Figure 2 below.

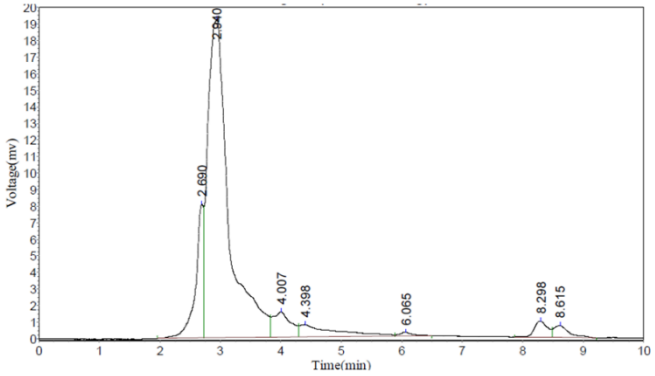

Fig. 2 : HPLC chromatogram for phenolic and flavonoid profile of the plant extract of wheatgrass.

In vitro Antioxidant Activity

The protective antioxidant ability of the aqueous plant extract was estimated in terms of *in vitro* free radical scavenging activity (using H_2O_2 and DPPH), inhibition of NO production and total reductive ability. Details are as shown in Figures 3-5. Results of this proved potential free radical scavenging activity of the aqueous plant extracts of *Triticum aestivum*.

As seen in Figure 3 below, antioxidant activity of the plant extract was compared with that of the standard antioxidant i.e., Butylated hydroxy anisol (BHA); the

inhibition rate is similar at 25 µg/ml. Although a larger difference is seen at 5 µg/ml, it is well within the range. The overall inhibition percentage of AET is seen to be similar to BHA, thus showing that the H_2O_2 radical scavenging activity of AET is similar to that of a standard antioxidant.

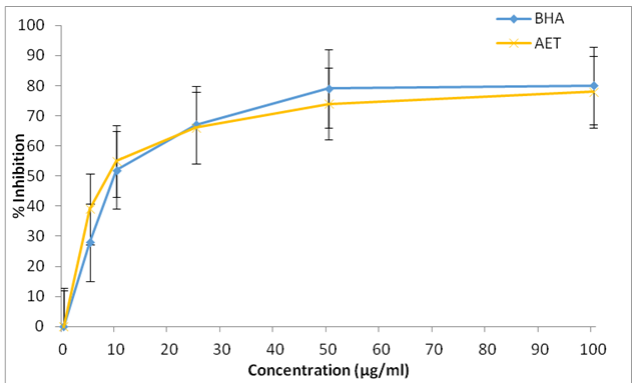

Fig. 3: H_2O_2 Radical Scavenging Activity

DPPH free radical scavenging activity of AET was measured using ascorbic acid as a positive control. Figure 4 represents the inhibition percentage of ascorbic acid and AET at different concentrations. It can be seen from the figure that, AET has a similar DPPH radical scavenging activity as ascorbic acid, thus proving that AET has valuable free radical scavenging activity.

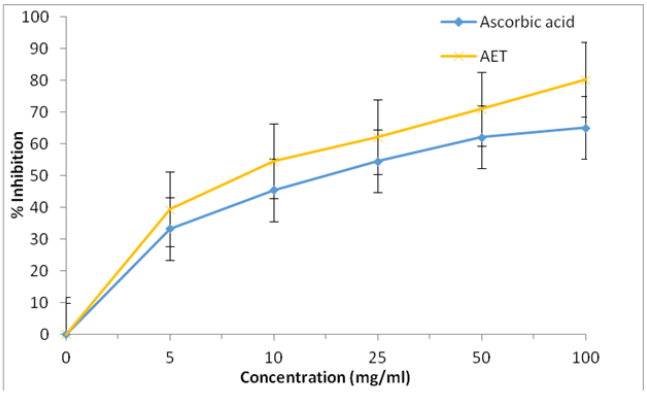

Fig. 4: DPPH Radical Scavenging Activity

A colorimetric assay was performed, to measure the NO radical scavenging activity of AET using ascorbic acid as a control. As seen in Figure 5, the results of the assay show that NO radical scavenging activity of AET is similar to that of a standard antioxidant.

Fig. 5: NO Radical Scavenging Activity

In vitro Antihemolytic Activity

Aqueous plant extract was tested for its antihemolytic efficacy. This study showed the antihemolytic ability of the aqueous plant extract. Data interpreted in Figure 6 indicated that the aqueous plant extract prevents H_2O_2 induced hemolysis.

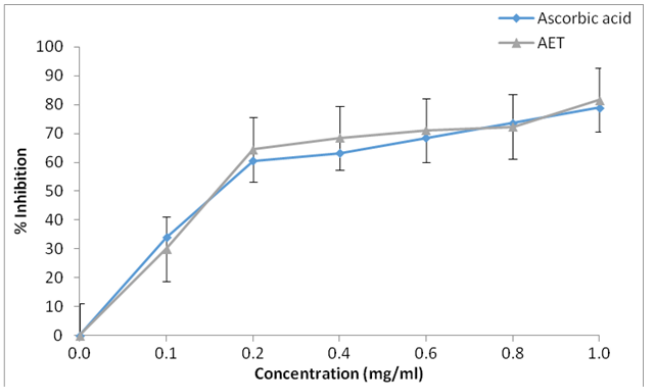

Fig. 6: Antihemolytic Activity

III. CONCLUSION

Free radical induced erythrocytic damage can be prevented through antioxidant and antihemolytic activity. In an attempt to curb such damage, properties of *Triticum aestivum* was explored. The present work clearly depicts that the plant *Triticum aestivum* is a rich repository of phytocompounds. These phytocompounds have been shown to be essential in scavenging free radicals and thus helping maintain the healthy cells in the body. HPLC analysis of the plant has shown the presence of a high concentration of polyphenols. *In vitro* colorimetric assays have proved that the plant has valuable antioxidant and antihemolytic properties. This has also paved way for further research on the therapeutic efficiency of *T. aestivum* against oxidative damage of not only blood but also other tissues and organs using animal and human cell line models.

IV. REFERENCES

[1]. Laufs U, Wassmann S, Czech T, Münzel T, Eisenhauer M, Böhm M, Nickenig G. Physical inactivity increases oxidative stress, endothelial dysfunction, and atherosclerosis. Arter. Thromb. Vasc. Biol. 2005; 25(4): 809-814.

[2]. Niki E, Komuro E, Takahashi M, Urano S, Ito E, Terao K. Oxidative hemolysis of erythrocytes and its inhibition by free radical scavengers. J. Biol. Chem. 1988; 263(36): 19809-19814.

[3]. Wu D, Cederbaum A I. Alcohol, oxidative stress, and free radical damage. Alcohol Res Health. 2003; 27: 277-284.

[4]. Aruoma O I. Free radicals, oxidative stress, and antioxidants in human health and disease. J. Am. Oil Chem.' Soc. 1998; 75(2): 199-212.

[5]. Julia P L, Buckberg G D, Acar C, Partington M T, Sherman MP. Studies of controlled reperfusion after ischemia. XXI. Reperfusate composition: superiority of blood cardioplegia over crystalloid cardioplegia in limiting reperfusion damage--importance of endogenous oxygen free radical scavengers in red blood cells. J Thorac Cardiovasc Surg. 1991; 101(2): 303-313.

[6]. Nagara B, District M. Antioxidant Activity of Indian Medicinal Plants. Indian J Nutr Diet. 2014; 51: 39.

[7]. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J. Biotechnol. 2006; 5(11): 1142-1145.

[8]. Mujoriya R, Bodla R. A study on wheat grass and its Nutritional value. Food Science and Quality Management.2011; ISSN 2225-0557

[9]. Ashok SA. Phytochemical and pharmacological screening of wheatgrass juice (Triticum aestivum L.). Int J Pharm Sci Rev Res. 2011; 9(1): 159-164.

[10]. Stubbendieck J, Hatch SL, Kjar KJ. North American range plants. University of Nebraska Press; 2nd ed. edition (August 1, 1982)

[11]. Sharma S, Shrivastav VK, Shrivastav A, Shrivastav BR, Dwivedi L. Therapeutic Potential Of Wheatgrass (Triticum Aestivum) Against Oxidative Stress By Platinium Containing Drugs During Cancer Chemotherapy: A Future Prospective. IMAJ. 2013; 1(5): 1-5

[12]. Singh N, Verma P, Pandey BR. Therapeutic potential of organic Triticum aestivum linn.(wheat grass) in prevention and treatment of chronic diseases: An overview. Int. J. Pharm. Sci. Drug Res. 2012; 4(1): 10-14.

[13]. Plaza L, de Ancos B, Cano PM. Nutritional and health-related compounds in sprouts and seeds of soybean (Glycine max), wheat (Triticum aestivum. L) and alfalfa (Medicago sativa) treated by a new drying method. Eur Food Res Technol. 2003; 216(2): 138-144.

[14]. Liyana Pathirana CM, Shahidi F. 2006. Antioxidant properties of commercial soft and hard winter wheats (Triticum aestivum L.) and their milling fractions.

[15]. J. Sci. Food Agric. 2006; 86(3): 477-485.

[16]. Padalia S, Drabu S, Raheja I, Gupta A. Dhamija M. Multitude potential of wheatgrass juice (Green Blood): An overview. Chron. young sci. 2010; 1(2): 23.

[17]. Kothari S. Jain A. K. Mehta S. C. Tonpay S. D. Hypolipidemic effect of fresh Triticum aestivum (wheat) grass juice in hypercholesterolemic rats. Acta Pol Pharm. 2011; 68(2) :291-294.

[18]. Shaikh M, Quazi M, Nandedkar R. Hypoglycemic Effect Of Wheatgrass Juice In Alloxan Induced Diabetic Rats. Pharma Tutor. 2011.

[19]. Jain G, Argal. Hepatoprotective potential of young leaves of Triticum Aestivum Linn. Against CCl4 induced hepatotoxicity. Int. J Pharm Sci Rev Res .2014; 5(11): 4751.

[20]. Arya, Priyanka, Madhu K. Chemoprevention by Triticum aestivum of mouse skin carcinogenesis induced by DMBA and croton oil-association with oxidative status. APJCP. 2011; 12 (1): 143-148.

[21]. Marwaha RK, Bansal D, Kaur S, Trehan A. Wheat grass juice reduces transfusion requirement in patients with thalassemia major: a pilot study. Indian Pediatrics. 2004; 41(7): 716-720.

[22]. Shyam R, Singh SN, Vats P, Singh VK, Bajaj R, Singh SB, Banerjee PK.Wheat grass supplementation decreases oxidative stress in healthy subjects: a comparative study with spirulina. J Altern Complement Med. 2007; 13(8): 789-792.

[23]. Ben-Arye E, Goldin E, Wengrower D, Stamper A, Kohn R, Berry E. Wheat grass juice in the treatment of active distal ulcerative colitis: a randomized double-blind placebo-controlled trial. Scand J Gastroenterol. 2002; 37(4): 444-449.

[24]. Aydos OS, Avci A, Özkan T, Karadağ A, Gürleyik E, Altinok B, Sunguroğlu A. Antiproliferative, apoptotic and antioxidant activities of wheatgrass (Triticumaestivum L.) extract on CML (K562) cell line. Turk J Med Sci, 2011; 41(4):657-663.

[25]. Bar-Sela G, Tsalic M, Fried G, Goldberg H. Wheat grass juice may improve hematological toxicity related to chemotherapy in breast cancer patients: a pilot study. Nutr Cancer. 2007; 58(1): 43-48.

[26]. Ashok Anup. Phytochemical and pharmacological screening of wheatgrass juice (Triticumaestivum L.) Int J Pharm Sci Rev Res.2011; 9(1): 159-164.

[27]. Priyabrata D, Suvra M, Sudeshna G, Kalipada D, Arpita G M, Swati D, Soma A M. Antioxidative and anticarcinogenic activities of methylpheophorbide a, isolated from wheat grass (Triticum Aestivum Linn.). Nat Prod Res. 2016; 30:4, 474-477.

[28]. Priyabrata D, Soma M, Suvra M, Abhijit C, Amartya P, Nirmal K S, Ashish M. Acute toxicity test of a natural iron chelator and an antioxidant, extracted from Triticum aestivum Linn. (Wheat grass). Nat Prod Res. 2014; 28(17) 1379-1382.

[29]. Kothari S, Jain, AK, Mehta, SC, Tonpay SD. Effect of fresh Triticum aestivum grass juice on lipid profile of normal rats. Indian J. Pharmacol. 2008; 40(5): 235.

[30]. Hebbani AV, Jamadagni MS, Chaitra M, Purnachandra KR, Singh A, Jyothy P, Pragathi V. Evaluation of In Vitro Radical Scavenging and Antihemolytic Efficacy of a Polyherbal Combination Prepared from Traditional Blood Purifiers. IJCRBP. 2016; 3(4):56-63.

[31]. Hebbani AV, Reddy VD, Nallanchakravarthula V. In Vitro Anti-hemolytic Activity of Terminalia arjuna (Roxb.) Wt. & Arn. Bark Powder Aqueous Extract.

[32]. Indian J Adv Chem Sci. 2014; 3:102-108.

[33]. Ak T, Gülçin İ. Antioxidant and radical scavenging properties of curcumin. Chem.-Biol. Interact. 2008; 174(1): 27-37.

[34]. Royer M, Diouf PN. Stevanovic T. Polyphenol contents and radical scavenging capacities of red maple (Acer rubrum L.) extracts. Food Chem Toxicol. 2011; 49(9): 2180-2188.

[35]. Fu W, Chen J, Cai Y, Lei Y, Chen L, Pei L, Zhou D, Liang X, Ruan J. Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from Parathelypteris nipponica (Franch. et Sav.) Ching. J Ethnopharmacol. 2010; 130(3): 521-528.

[36]. Girish TK, Vasudevaraju P, Rao UJP. Protection of DNA and erythrocytes from free radical induced oxidative damage by black gram (Vigna mungo L.) husk extract. Food Chem Toxicol. 2012: 50(5): 1690-1696.