Asian Pac J Trop Med 2014; 7(Suppl 1): S398-S404

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi: 10.1016/S1995-7645(14)60265-0

Phytochemical screening and analysis of antioxidant properties of aqueous extract of wheatgrass

Varalakshmi Durairaj, Muddasarul Hoda, Garima Shakya, Sankar Pajaniradje Preedia Babu, Rukkumani Rajagopalan*

Department of Biochemistry and Molecular Biology, School of Life Sciences, Pondicherry University, Puducherry, India

ARTICLE INFO

Article history:
Received 27 Apr 2014
Received in revised form 19 May 2014
Accepted 23 Jun 2014
Available online 21 Aug 2014

Keywords:
Antioxidant
GC-MS
Total polyphenol
Flavanoid
Wheatgrass

ABSTRACT

Objective: To screen the phytochemical constituents and study antioxidant properties of the aqueous extract of the wheatgrass.

Methods: The current study was focused on broad parameters namely, phytochemical analysis, gas chromatography-mass spectrometry analysis and antioxidant properties in order to characterize the aqueous extract of wheatgrass as a potential free radical quencher.

Results: The phytochemical screening of the aqueous extract of wheatgrass showed the presence of various secondary metabolites but the absence of sterols and quinone in general. Wheatgrass was proved to be an effective radical scavenger in all antioxidant assays. The gas chromatography-mass spectrometry analysis confirmed the presence of diverse category of bioactive compounds such as squalene, caryophyllene and amyrins in varying percentage.

Conclusions: From the results obtained, we conclude that wheatgrass aqueous extract contains various effective compounds. It is a potential source of natural antioxidants. Further analysis of this herb will help in finding new effective compounds which can be of potent use in pharmacological field.

1. Introduction

Oxidative stress is a condition where the homeostasis between the oxidants and antioxidants are disturbed with inclination of more oxidative species being generated than the amount of counter-oxidative species being produced in defence. Endogenous oxidants such as superoxide anion, hydrogen peroxide and hydroxyl radicals are few of the major players that induce oxidative stress resulting from normal cellular metabolism[1]. Failure of clearance of these oxidants due to one or more reasons may lead to oxidative stress condition, thus resulting in serious damage to the DNA, proteins and other subcellular components[2,3]. The consequences of mild to severe oxidative stress have been reported to be the major causative source of several diseases like cancer, diabetes, hypertension, atherosclerosis, acute respiratory distress syndrome, *etc*[3-5].

Wheatgrass is grown from common wheat (*Triticum aestivum*), a subspecies of the family Poaceae[6]. It is widely grown throughout temperate regions of North America and Europe. Indigenously, wheatgrass has been used as an herbal medicine since ages. In 1930s Charles F Schnabel brought wheatgrass for experimental trails[7]. Ever since it has been manufactured and marketed by various consumer-based product companies. Being a non-toxic herb it also evades Food and Drug Administration screening and clearance. It has been traditionally used as a herbal medicine in a number of serious diseases like thalassemia and myelodysplastic syndrome[8,9]. In addition, it has also been believed to

*Corresponding author: Dr. Rukkumani Rajagopalan, Assistant Professor, Department of Biochemistry and Molecular Biology, School of Life Sciences, Pondicherry University, Puducherry-605014, India.
Tel: +91 413-2654537, +91 9677847337
E-mail: ruks2k2@yahoo.co.in, rrlabs2011@gmail.com

strengthen the immune system and increase the life span of cancer patients by regressing the spread of cancer cells[10]. In the present study, we have focused on broad research areas namely, phytochemical analysis, gas chromatography-mass spectrometry (GC-MS) analysis, antioxidant ability and antiproliferative property in order to understand the potential of wheatgrass as an oxidative stress quencher. Information on the qualitative and quantitative compositions and antioxidant property of aqueous extract of wheatgrass would provide scientific basis to justify its therapeutic use.

In the current study, water was used as the solvent to extract the antioxidants with hydrophilic properties. For the plants used in food, extracts made with water are nutritionally more relevant as they have an edge over other solvents when it comes to certification and safety[10]. Recently, there was a major concern about contaminations due to solvent usage in the preparation of plant extracts. To minimize this, traditional extracts have always been prepared as decoctions of aqueous extracts[11]. Wheatgrass fresh juice has long been used as a nutritional supplement[12-14]. In this view the present study has been carried out with aqueous extract of wheatgrass. In this study we aimed at analysing the presence of natural antioxidants in aqueous extract and validating the use of wheatgrass in traditional medicines.

2. Materials and methods

2.1. Sample preparation

A total of 30 g of dried powder, manufactured by Eden Park Agro Products Pvt. Ltd., Pune, India was extracted with 300 mL water using Soxholet apparatus for 24 h. The aqueous extract was lyophilized and stored in 4 °C.

2.2. Total polyphenol content

Total polyphenol content of this aqueous extract was analysed using Folin's Ciocalteau reagent according to the protocol designed by Singleton *et al*[15]. Briefly, 1 mL of sample of varying concentrations was incubated in 5 mL of Folin-Ciocalteu reagent and 4 mL of 1 mol/L Na_2CO_3. After 15 min of incubation, absorbance was measured at 765 nm by spectrophotometer (Shimadzu UV-1700). Gallic acid dissolved in 50% ethanol was used as standard. The total polyphenol content was reported in terms of μmole of gallic acid equivalents/g of extract (GAEs).

2.3. Total flavonoid content

The total flavonoid content was estimated by the method developed by Jia *et al*[16]. Briefly, 0.3 mL of 5% (w/v) $NaNO_2$ was added to every 4 mL of extract solution of varying concentrations. After 5 min of incubation, 0.3 mL of 10% (w/v) $AlCl_3$ was added. After 6 min, 2 mL of 1 mol/L NaOH was added. The total volume was made up to 10 mL with distilled water. After vortex shaking for 1 min, the absorbance was noted at 510 nm. The total flavonoid content was reported in terms of μmole of quercetin equivalents/g of extract.

2.4. Phytochemical screening

Phytochemical screening for the presence of major types of compounds present in the extract was done by standard methods developed by Harbone[17].

2.5. Antioxidant properties

2.5.1. Ferric reducing power

It was carried out according to Barros *et al*[18]. Briefly, 2.5 mL of extract of varying concentrations were incubated at 50 °C for 20 min together with 2.5 mL of 200 mmol/L of Na-phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. After incubation, 2.5 mL of 10% trichloroacetic acid was added to the reaction mixture and centrifuged at 1 000 r/min for 8 min. About 5 mL of supernatant was extracted and made up to 10 mL with deionized water. To this solution, 1 mL of 0.1% ferric chloride was added and absorbance was measured at 700 nm. Butylated hydroxyanisole (BHA) was used as a standard.

2.5.2. Metal chelating activity

Metal chelating activity was estimated by the protocol as described previously by Chan *et al*[19]. Briefly, 1 mL of various concentrations of the extract was incubated for 10 min together with equal volume of $FeSO_4$ (0.1 mmol/L) and ferrozine (0.25 mmol/L) with vortexing. The absorbance was measured at 562 nm. The % chelating activity was calculated by the following formula:

Metal chelating ability (%)=$[(A_0-A_1)/A_0]\times100$

Where A_0=absorbance of the control; A_1=absorbance of the sample/ standard. Butylated hydroxytoluene (BHT) was taken as a positive control.

2.5.3. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined according to the method described by Klein *et al*[20]. About 1 mL of various wheatgrass extracts (ranging from 0.2 to

1.0 mg/mL) were added to the tubes and mixed with 1 mL of iron-ethylene diamine tetraacetic acid (EDTA) solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL of EDTA (0.018%), and 1 mL of dimethyl sulfoxide (0.85% v/v in 0.1 mol/L phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 mL of 0.22% ascorbic acid. Test tubes were tightly capped and heated on a water bath at 80-90 °C for 15 min. The reaction was terminated by the addition of ice-cold trichloroacetic acid (17.5% w/v). About 3 mL of Nash reagent was added to all the tubes and incubated at room temperature for 15 min for colour development. Varying intensities of yellow colour formed was measured at 412 nm against reagent blank. Percentage hydroxyl radical scavenging was calculated by the formula as mentioned in metal chelating activity. BHT was used as the standard.

2.5.4. Hydrogen peroxide scavenging activity

The ability of wheatgrass extracts to scavenge hydrogen peroxide was determined by the method of Rosen and Rauckman[21]. About 1 mL of various wheatgrass extracts (ranging from 0.2 to 1.0 mg/mL) was dissolved in 3.4 mL of 0.1 mol/L phosphate buffer (pH 7.4) and mixed with 600 μL of 43 mmol/L solution of hydrogen peroxide. The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extracts and a standard compound (BHT) was calculated by the formula mentioned in metal chelating ability.

2.5.5. Nitric oxide scavenging activity

Nitric oxide was generated from sodium nitroprusside and its quantity was determined using Griess reagent according to the method of Sousa *et al*[22]. Briefly, 100 μL of extract solution at different concentrations (ranging from 0.2 to 1.0 mg/mL of solvent) was mixed with 100 μL of sodium nitroprusside solution (20 mmol/L), 100 μL Griess reagent (1% sulphanilamide in 2% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance was measured at 562 nm after 10 min of incubation at room temperature. The percentage of nitric oxide scavenging by the wheatgrass extracts was calculated by the formula mentioned in metal chelating ability. BHA was used as the standard.

2.5.6. Total antioxidant activity

The antioxidant activity of the wheatgrass extracts was evaluated by the phosphomolybdenum method according to the procedure previously reported by Prieto *et al*[23]. Briefly, 0.3 mL of various concentrations of wheatgrass extracts (ranging from 0.2 to 1.0 mg/mL) was mixed with a 3 mL solution containing 0.6 mol/L sulphuric acid, 28 mmol/L sodium phosphate and 4 mmol/L ammonium molybdate followed by incubation at 95 °C for 90 min. After cooling to 25 °C, absorbance of the resulting solution was measured spectrophotometrically at 695 nm. Ascorbic acid was used as the positive control.

2.6. GC-MS analysis

GC-MS analysis was carried out at Sargam Laboratories Pvt. Ltd., Chennai, India, under the supervision of the technical analyst. The qualitative analysis was done by the standard method of Harbone[17].

2.7. Statistical analysis

The results are presented as mean±SD of triplicate observations. All the data were analyzed using the SPSS 13-Windows Students version software. Statistical analysis was done by analysis of variance (ANOVA) followed by Tukey's test. $P \leqslant 0.05$ was considered to be statistically significant.

3. Results

In the present study, aqueous extract of wheatgrass was screened for the presence of bioactive compounds. The results showed the presence of alkaloids, flavonoids, saponin, tannins, amino acids and protein, carbohydrates, coumarin, phenols, alkaloids, terpenoids and cardiac glycosides. But, sterol, steroids and quinone were absent in aqueous extract (Table 1).

Table 1
Phytochemicals studies of aqueous extracts of wheatgrass.

Particulars	Aqueous extract
Alkaloids	++
Flavonoids	+++
Tannins	++
Amino acid and protein	++
Carbohydrate	+
Cardioglycoside	++
Saponins	+
Coumarin	++
Terpenoids	++
Sterol and steroids	–
Quinone	–

+: positive; –: negative.

Aqueous extract of wheatgrass was evaluated quantitatively

for the percentage composition of total phenolic and flavonoids. The total phenolic content was found to be (210.15 ±2.14) μmol of GAE/g equivalent of wheatgrass. While, the total flavonoid content was found to be (160.25±2.17) μmol of quercetin/g equivalent of wheatgrass.

The antioxidant activity of wheatgrass extract was determined by measuring its ability to reduce ferric to ferrous ion. The reducing power was confirmed by the changes of yellow colour to green and blue shades. Wheatgrass extract had higher antioxidant activity compared to BHA, the reference compound (Figure 1A). The metal chelating activity of the plant extract was comparable to that of BHT (Figure 1B). Wheatgrass extract quenched hydroxyl radical in a concentration dependent manner and a comparable scavenging activity was observed between the extract and the standard drug (BHT) (Figure 1C). Wheatgrass extract significantly reduced the hydrogen peroxide with increase in concentration. The percentage inhibition of the hydrogen peroxide correlated with results of BHT (Figure 1D). The scavenging activity of the extract against nitric oxide released by sodium nitroprusside was investigated with the help of standard BHA (Figure 1E). Wheatgrass extract showed high inhibitory activities at all the concentrations tested and the total antioxidant activity of wheatgrass extract was comparable to the standard compound ascorbic acid used in the present study (Figure 1F).

The GC-MS analysis of wheatgrass aqueous extract showed the presence of many bioactive compounds and contained various pharmacologically important compounds like gamma sitosterol, squalene, caryophyllene and amyrins (Table 2).

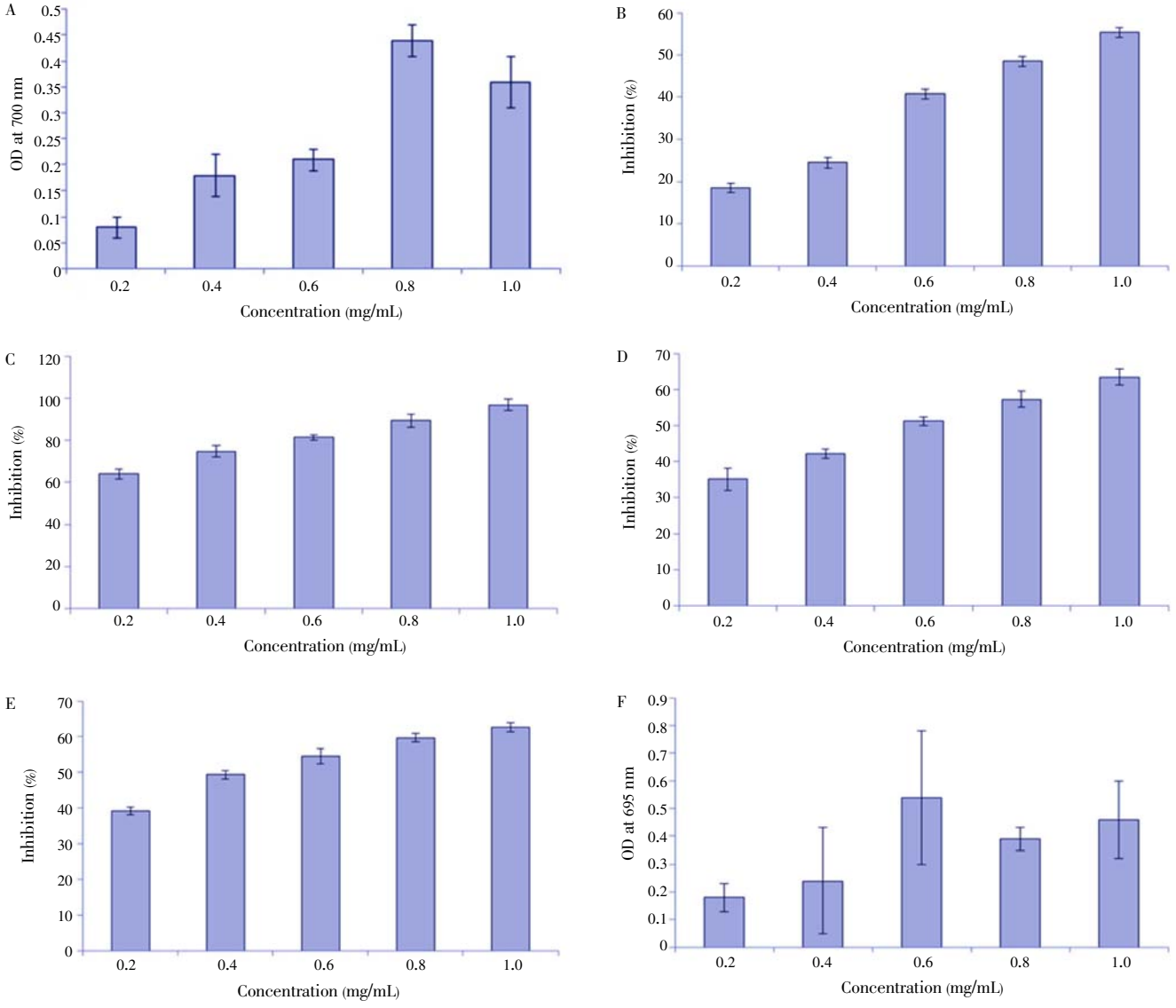


Figure 1. Results of ferric reducing power (A), metal chelating activity (B), hydroxyl radical scavenging activity (C), hydrogen peroxide radical scavenging activity (D), nitric oxide radical scavenging activity (E) and total antioxidant activities (F).
Values are mean±SD of triplicate observation in each group. ANOVA followed by Tukey's test.

Table 2

GC-MS analysis of the aqueous extract.

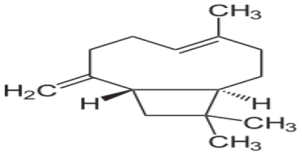
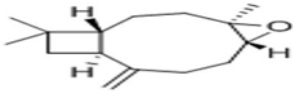
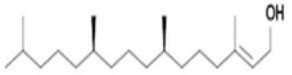
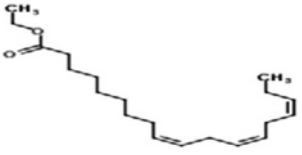
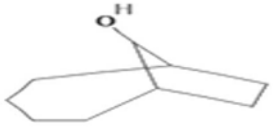
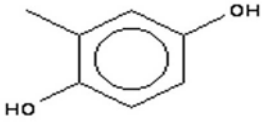
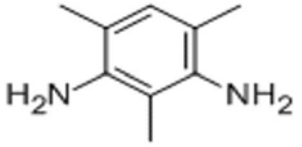
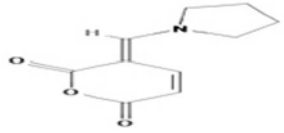
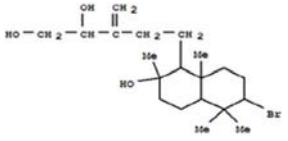
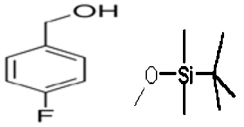
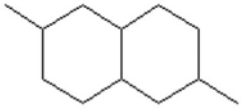
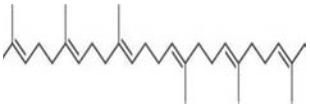
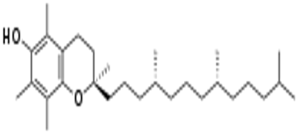
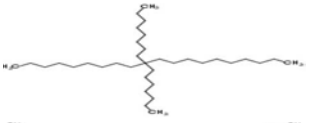
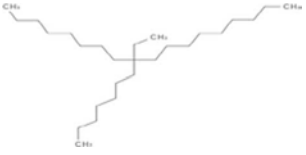
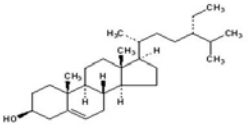
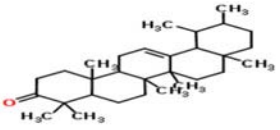
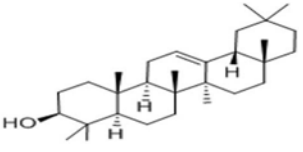
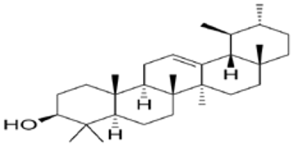
Compound name	Percent (%)	Structure of the compound
Caryophyllene (sesquiterpene)	0.91	
Caryophyllene oxide (sesquiterpene)	1.88	
Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)- (cis-Pinnane)	2.41	
n-Hexadecanoic acid (saturated fatty acid)	5.33	
Phytol (acyclic diterpene alcohol)	0.98	
9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (polyunsaturated fatty acid)	12.57	
Ethyl9,12,15-octadecatrienoate (ethyl ester)	1.33	
syn-9-Hydroxybicyclo [4.2.1] nonane (alcohol)	1.33	
1,4-Benzenediol, 2-methyl- (alcohol)	1.02	
1-Nonadecene (unsaturated hydrocarbon)	0.97	
2,4,6-Trimethyl-1,3-phenylenediamine (diamine)	3.21	
N,N-Tetramethylene-.alpha.-(aminomethylene) glutaconic anhydride	22.58	
1,2-Pentanediol, 5-(6-bromodecahydro-2-hydroxy-2,5,5a,8a-tetramethyl-1-naphthalenyl)-3-methylene-	4.54	

Table 2, continued

GC-MS analysis of the aqueous extract.

Compound name	Percent (%)	Structure of the compound
4-Fluorobenzyl alcohol, tert-butyldimethylsilyl ether	1.33	
Silane, chlorodiethyl(dodec-9-ynyloxy)-(Ester)	0.98	
Naphthalene, decahydro-2,6-dimethyl-(cyclohydrocarbon)	1.14	
2-methyloctacosane (hydrocarbon)	2.52	
Squalene (polyunsaturated hydrocarbon)	3.81	
Nonacosane (hydrocarbon)	3.78	
Triacontane (hydrocarbon)	3.64	
dl-.alpha.-Tocopherol (methylated phenol)	3.86	
Eicosane, 10-heptyl-10-octyl-	1.01	
Octadecane, 9-ethyl-9-heptyl-	3.70	
gamma.-Sitosterol	3.71	
4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	2.43	
beta.-Amyrin	0.99	
alpha.-Amyrin	4.02	

4. Discussion

Though, traditionally wheatgrass has been used for various disorders, till date, not much scientific literature is available on wheatgrass. It clearly signifies that a whole lot of scientific exploration needs to be done on this herb which could give lead for natural alternative for diverse diseases against synthetic drugs. Demand for phytomedicine is on the rise because of its minimal side effects.

Oxidative stress has been an age old problem and intensive research has been done for quite some time. Oxidative stress seems to be one of the fundamental disorders which tend to be the mother of other disorders such as diabetes, hypertension, cancer and ulcer. If by any means we are successful in tackling the oxidative stress and able to keep it at bay by some natural, zero side-effect alternative, we would be successful in countering many other follow up diseases and disorders. Precisely with this hypothesis, we tested the antioxidant property of wheatgrass, thus confirming its ability to counter the excessive radicals of all major types. The reducing power of the extract was comparable with BHA and the related compound BHT and ascorbic acid which were used as standard compounds in concentration dependent manner. The results exhibited a positive linear correlation between the concentration and the free radical scavenging activities.

The total phenolic and flavonoid content shows significant presence of polyphenols and other flavonoids as confirmed by GC-MS analysis. GC-MS analysis confirmed diverse class of organic compounds in varying percentage present in the aqueous extract, ranging from saturated and unsaturated fatty acids (such as *n*-hexadecanoic acid and octadecatrienoic acid), alcohols (such as phytol and 2-methyl benzenediol), terpenes (such as alpha and beta amyrin, caryophyllene and caryophyllene oxide). Majority of the compounds extracted belong to hydrocarbon class such as octadecene, nonadecene, 2-methyl octacosane, squalene, nonacosane and tricontane. Sterols such as gamma sitosterol are also reported in GC-MS analysis. Each of these compounds influence metabolism in some way or the other. Gamma sitosterol has been reported to influence cholesterol synthesis in liver and intestinal cell lines[24]. It has also been reported to act as a cytotoxic sensitizing agent[25]. Squalene is a polyunsaturated hydrocarbon that has been reported to prevent oxidative damage to the striatum in a mouse while also controlling the toxicity of 6-hydroxydopamine[26]. Octadecane, another hydrocarbon present in significant amount in the water extract is reported to have shown significant reduction in pathophysiology in *Plasmodium berghei* infected animals when treated with extracts of *Artemisia khorasanica* that contains this particular compound in significant concentrations[27]. Caryophyllene and its oxides are one of the most important biological compounds that influences diverse metabolism. Caryophyllene has been reported to show significant anti-cancer properties. It induces apoptosis through suppression of multiple pathways such as P13K, AKT, mTOR and S6K1[28]. Many more effects like anti-bacterial, anti-fungal, immunomodulatory and anti-inflammatory have also been reported to show links with caryophyllene oxides[29]. Anti-platelet aggregation activity has also been reported *in vitro*[30]. Alpha and beta amyrin are two biologically active pentacyclic triterpenes that influence wide physiological parameters such as anti-inflammatory, antioxidant, gastroprotective and hepatoprotective effects at non-toxic concentrations[31-33]. Anti-hyperglycemic effects and hypolipidemic effects of amyrin have been recently investigated with positive conclusion suggesting that the compound has a potential for drug development for diabetes and atherosclerosis[34].

Thus, the GC-MS analysis report of the water extract of wheatgrass clearly shows the presence of some biologically functional compounds which has been reported to have multiple effects in regulating the general physiological and biochemical parameters, rightly justifying why the wheatgrass has been a famous home-made remedy for a number of diseases since the ancient times, to this day in many tribal and traditionally bound societies.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors thank DST-FIST, UGC-SAP, DBT-IPLS for providing the infra structural facility.

References

[1] Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. *World Allergy Organ J* 2012; **5**: 9-19.

[2] Halliwell B, Gutteridge JMC. *Free radicals in biology and medicine*. New York: Oxford University Press; 1999.

[3] Marnett LJ. Lipid peroxidation DNA damage by malondialdehyde. *Mutat Res* 1999; **424**: 83-95.

[4] Kukreja RC, Hess ML. The oxygen free-radical system: from equations through membrane-protein interactions to cardiovascular injury and protection. *Cardiovasc Res* 1992; **26**(7): 641-655.

[5] Andreadis AA, Hazen SL, Comhair SA, Erzurum SC. Oxidative

and nitrosative events in asthma. *Free Radic Biol Med* 2003; **35**(3): 213-225.

[6] Padalia S, Drabu S, Raheja I, Gupta A, Dhaija M. Multiple potential of wheatgrass juice (green blood): an overview. *J Postgrad Med* 2010; **1**: 23-28.

[7] Murphy S. Wheatgrass, healthy for the body and the bank account. ABC Landline; 2002. [Online] Available from: http://www.abc.net.au/landline/stories/s689970.htm [Accessed on 17th July, 2014]

[8] Marawaha RK, Bansal D, Kaur S, Trehan A. Wheatgrass juice reduces transfusion requirement in patients with thalassemia major: a pilot study. *Indian Pediatric* 2004; **41**(7): 716-720.

[9] Mukhopadhyay S, Basak J, Kar M, Mandal S, Mukhopadhyay A. The role of iron chelation activity of wheat grass juice in patients with myelodysplastic syndrome. *J Clin Oncol* 2009; **27**: S15.

[10] Moller JKS, Madsen HL, Aaltonen T, Skibsted LH. Dittany (*Origanum dictamnus*) as a source of water-extractable antioxidant. *Food Chem* 1999; **64**: 215-219.

[11] Rahim ZH, Khan HB. Comparative studies on the effect of crude aqueous (CA) and solvent (CM) extracts of clove on the carcinogenic properties of *Streptococcus mutans*. *J Oral Sci* 2006; **48**(3): 117-123.

[12] Shakya G, Madhu Sudhana Rao K, Sankar P, Rukkumani R. Antihyperglycaemic and antihyperlipidaemic effect of wheatgrass on streptozotocin induced type 2 diabetic rats. *J Pharm Res* 2012; **5**(5): 2580-2583.

[13] 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**: 235-236.

[14] Alitheen NB, Oon CL, Keong YS, Chuan TK, Li HK, Yong HW. Cytotoxic effects of commercial wheatgrass and fiber towards human acute promyelocytic leukemia cells (HL60). *Pak J Pharm Sci* 2011; **24**(3): 243-250.

[15] Singleton VL, Rossi JA Jr. Colorimetric of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 1965; **16**: 144-158.

[16] Jia Z, Tang M, Wu J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* 1999; **64**: 555-559.

[17] Harbone AJ. *Phytochemical methods: a guide to modern technique of plant analysis*. New York: Chapman Hall; 1973.

[18] Barros L, Baptista P, Ferreira IC. Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays. *Food Chem Toxicol* 2007; **45**(9): 1731-1737.

[19] Chan EWC, Lim YY, Omar M. Antioxidant and antibacterial activity of leaves of *Etlingera* species (Zingiberaceae) in Peninsular Malaysia. *Food Chem* 2007; **104**(4): 1586-1593.

[20] Klein SM, Cohen G, Cederbaum AI. Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system. *Biochemistry* 1981; **20**(21): 6006-6012.

[21] Rosen GM, Rauckman EJ. Spin trapping of superoxide and hydroxyl radicals. *Methods Enzymol* 1984; **105**: 198-209.

[22] Sousa C, Valentão P, Ferreres F, Seabra RM, Andrad PB. Tronchuda cabbage (*Brassica oleracea* L. var. costata DC)-scavenger of reactive nitrogen species. *J Agric Food Chem* 2008; **56**(11): 4205- 4211.

[23] Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem* 1999; **269**(2): 337-341.

[24] Ho SS, Pal S. Margarine phytosterols decrease the secretion of atherogenic lipoproteins from HepG2 liver and Caco2 intestinal cells. *Atherosclerosis* 2005; **182**(1): 29-36.

[25] Carter BA, Taylor OA, Prendergast DR, Zimmerman TL, Von Furstenberg R, Moore DD, et al. Stigmasterol, a soy lipid-derived phytosterol, is an antagonist of the bile acid nuclear receptor FXR. *Pediatr Res* 2007; **62**: 301-306.

[26] Kabuto H, Yamanushi TT, Janjua N, Takayama F, Mankura M. Effects of squalene/squalane on dopamine levels, antioxidant enzyme activity, and fatty acid composition in the striatum of Parkinson's disease mouse model. *J Oleo Sci* 2003; **62**(1): 21-28.

[27] Nahrevanian H, Esmaeili B, Kazemi M, Nazem H, Amini M. *In vivo* antimalarial effects of Iranian flora *Artemisia khorassanica* against *Plasmodium berghei* and pharmacochemistry of its natural components. *Iran J Parasitol* 2009; **5**(1): 6-19.

[28] Park KR, Nam D, Yun HM, Lee SG, Jang HJ, Sethi G, et al. β-Caryophyllene oxide inhibits growth and induces apoptosis through the suppression of PI3K/AKT/mTOR/S6K1 pathways and ROS-mediated MAPKs activation. *Cancer Lett* 2011; **312**: 178-188.

[29] Astani A, Reichling J, Schnitzler P. Screening for antiviral activities of isolated compounds from essential oils. *Evid Based Complement Altern Med* 2011; doi: 10.1093/ecam/nep187.

[30] Lin WY, Kuo YH, Chang YL, Teng CM, Wang EC, Ishikawa T, et al. Anti-platelet aggregation and chemical constituents from the rhizome of *Gynura japonica*. *Planta Med* 2003; **69**: 757-764.

[31] Oliveira FA, Chaves MH, Almeida FR, Lima RC Jr, Silva RM, Maia JL, et al. Protective effect of alpha- and beta-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. trunk wood resin, against acetaminophen-induced liver injury in mice. *J Ethnopharmacol* 2005; **98**(1-2): 103-108.

[32] Holanda Pinto SA, Pinto LM, Cunha GM, Chaves MH, Santos FA, Rao VS. Anti-inflammatory effect of alpha, beta-amyrin, a pentacyclic triterpene from *Protium heptaphyllum* in rat model of acute periodontitis. *Inflammopharmacology* 2008; **16**(1): 48-52.

[33] Aragão GF, Cunha Pinheiro MC, Nogueira Bandeira P, Gomes Lemos TL, de Barros Viana GS. Analgesic and anti-inflammatory activities of the isomeric mixture of alpha and beta-amyrin from *Protium heptaphyllum* (Aubl.) march. *J Herb Pharmacother* 2007; **7**(2): 31-47.

[34] Santos FA, Frota JT, Arruda BR, de Melo TS, da Silva AA, Brito GA, et al. Antihyperglycemic and hypolipidemic effects of α, β-amyrin, a triterpenoid mixture from *Protium heptaphyllum* in mice. *Lipids Health Dis* 2012; **11**: 98.