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ORIGINAL RESEARCH

Comparative Assessment of the Antioxidant Activities, Total Phenolics and Fatty Acid Composition in Three Indian Oats Varieties

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ABSTRACT

A comparative assessment of the total phenolic content and antioxidant activity of polar extracts of three Indian varieties (SKO 90, Sabzaar and SKO 96) of oats (*Avena sativa* L.) was carried out. Acidified aqueous ethanolic extracts of oats were sequentially extracted with hexane, ethyl acetate, n-butanol and water to obtain four fractions in each variety. In all varieties, weights of different fractions followed the order: Hexane>water>n-butanol>ethyl acetate. The hexane fraction of all the three varieties was analyzed for lipid composition by gas chromatography. All fractions were evaluated for total phenolic content by the Folin-Ciocalteu method and antioxidant activity using three *in vitro* assays, namely DPPH, ABTS and Nitric Oxide scavenging assays. Quantitative estimation of fatty acids in the hexane-soluble fraction of all the three varieties demonstrated that all the varieties had similar fatty acid composition. However, the antioxidant assays demonstrated that the SKO 90 variety had better antioxidant potential followed by SKO 96 and Sabzaar.

KEY WORDS: Oats, fractionation, total phenol content, antioxidant, Gas Chromatography.

INTRODUCTION

Oats (*Avena sativa* L.) has been recognized as a healthy food for a long time, owing to its dietary benefits and nutritional value. Oats contain unique low molecular weight phenolic compounds called avenanthramides (Avns) besides many other phytochemicals such as tocotrienols, phenolic acids, flavanoids, sterols and phytic acid which are reported to contribute to their strong *in vitro* and *in vivo* antioxidant activity (D. M. Peterson, Hahn, and Emmons, 2002). Current evidence suggests that consumption of foods containing oats attenuated exercise-induced production of reactive oxygen species (ROS) prevented coronary heart disease, improved symptoms of diabetes, and obesity (Berg, König, Deibert,

and Grathwohl, 2003; Dong, Cai, Shen, and Liu, 2011; Koenig *et al.*, 2014; Zdunczyk, Flis, and Zielinski, 2006). Cell culture studies have also revealed antiproliferative effects of oats Avns on vascular smooth muscle cells and colon cancer cells thereby reducing the risk for the development of colon cancer and atherosclerosis respectively (Guo *et al.*, 2010; Libby, Ridker, and Maseri, 2002).

Antioxidant activities in oats grain are highly correlated with its total polyphenols which is greatly influenced by genotype and the growing environment (Emmons and Peterson, 2001; D. Peterson, Wesenberg, and Burrup, 2005). Earlier studies have reported the relationship between oats major nutritional

components and contents of total polyphenols and the Chinese naked oats (Tong *et al.*, 2014). The correlation between avenanthramide content and chemical composition of four Mexican oat varieties was also reported (Ortiz *et al.*, 2013). Bryngelsson *et al.* studied lipids and antioxidants in the groats and hulls of seven Swedish oat varieties (Bryngelsson, Mannerstedt-Fogelfors, Kamal-Eldin, Andersson, and Dimberg, 2002). One of the studies reported the effect of toasting on antioxidant properties of flours from different oats cultivars grown in India (Sandhu, Godara, Kaur, and Punia, 2015).

To the best of our knowledge there is no study on assessment of total phenolics, antioxidant potential and total lipid fatty acid composition in Indian varieties of oats. Screening of a more diverse population would be desirable to select from among an elite group of genotypes those with the most favorable (antioxidant) characteristics. Hence, the objective of the present study was to make a comparative assessment of total phenolics, antioxidant activity and total lipid fatty acid composition of three national release Indian varieties of oats (SKO 90, Sabzaar and SKO 96).

RESULTS AND DISCUSSION

The moisture content of the oats was determined gravimetrically and was found to be in the range of 2.10-2.23%. The whole grain oats (10 g) was extracted with acidified aqueous ethanol (100 ml) to get the ethanol-soluble polar extract, which corresponds to nearly 7.3-8.5% (on dry basis) among three varieties of the oats. The sequential fractionation of the acidified aqueous ethanol extract with different solvents in order of increasing polarity of hexane, ethyl acetate, n-butanol, and water led to four fractions, namely, HEX, EA, BUT and WAT as shown in Table 1. The percentage of hexane soluble in all varieties is found to be in the range of 69.6-73.9%, followed by water-soluble (13.7-

difference in antioxidant capacity among different varieties of 17.4%), butanol-soluble (4.8-9.6%) and ethyl acetate-soluble (4.1-5.7%). Our results are in line with those reported previously (Cai *et al.*, 2011).

The TLC profile of hexane-soluble in 80:20, v/v of hexane: ethyl acetate indicated the presence of partial glycerides (diacylglycerol), free fatty acids and traces of triglyceride. Total glycerides were derivatized to fatty acid methyl ester and analyzed by GC in order to have a comparative fatty acid composition among three varieties of oats (Table 2). Chromatographic results showed the presence of both saturated and unsaturated fatty acids with linoleic acid (18:2) being the major fatty acid present (35-39%) followed by oleic acid (18:1) (30-32.3%) in all the three varieties of oats. All three Indian varieties possess very similar fatty acid profile as shown in Figure 1. The fatty acid composition matched with those reported in the literature (Brindzová *et al.*, 2008).

The total phenolic content (TPC) of different fractions, as determined by Folin-Ciocalteu method is shown in Table 3. Results demonstrated that EA fraction of all the three varieties contained significantly ($p < 0.001$) higher amount of total phenolics compared to other fractions and followed the order: EA>BUT>WAT>HEX. This suggests that EA fraction is a phenol-enriched fraction matching well with earlier literature report (Cai *et al.*, 2011). Among the three varieties, SKO 90 contained significantly ($p < 0.01$) higher amount of total phenolics compared to other varieties.

All fractions of three different varieties of oats were also evaluated for antioxidant potency using the three well known *in vitro* assays, namely DPPH, ABTS and nitric oxide radical-scavenging assays. Similar to TPC, the EA fraction of all the three oats varieties had the strongest DPPH radical scavenging activity, followed by the n-butanol fraction.

Table 1: Weights of different fractions of three varieties of oats

Varieties	Weight of polar extract (g)	% dry basis	Weights of different fractions (mg)			
			HEX	EA	BUT	WAT
SKO 90	0.72±0.03	7.4	253.3±21.6 [69.6]	20±2.8 [5.5]	35±12.2 [9.6]	55.7±3.3 [15.3]
SABZAAR	0.72±0.03	7.3	244±13.2 [72.1]	19.2±2.9 [5.7]	16.2±1.2 [4.8]	58.8±2.6 [17.4]
SKO 96	0.84±0.05	8.5	281.3±17.9 [73.9]	15.5±1.4 [4.1]	31.5±2.3 [8.3]	52.2±3.8 [13.7]

*Values in parenthesis indicate percentage of extracts

Table 2: Comparative fatty acid composition of hexane-soluble fractions of three varieties of oats

Fatty acid	Composition (in wt%)		
	SKO 90	SABZAAR	SKO 96
14:0	0.80	0.50	0.70
16:0	24.0	22.9	24.6
16:1	1.10	0.20	0.40
18:0	2.20	2.80	2.50
18:1	32.3	31.9	30.0
18:2	35.9	39.0	38.2
18:3	1.70	1.30	1.40
20:0	0.30	0.20	0.40
20:1	0.80	0.60	0.60
22:0	0.30	0.20	0.50
24:0	0.50	0.40	0.60

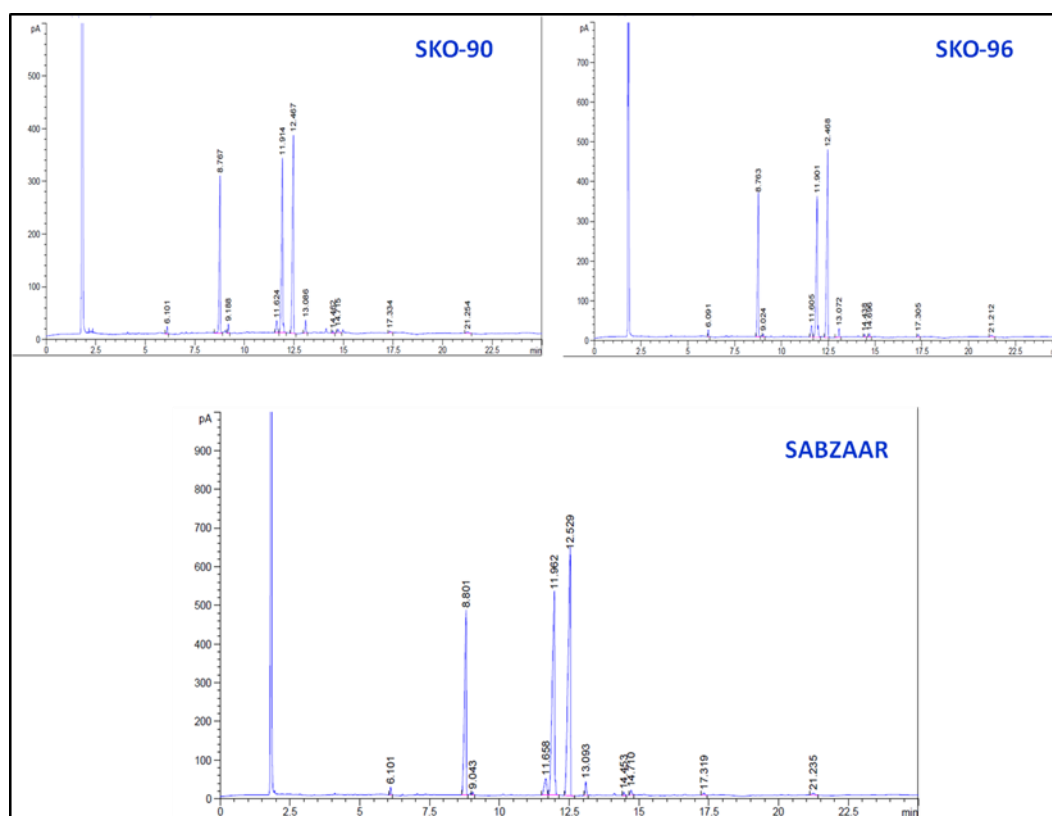


Figure 1: Gas Chromatograms of hexane extracts of SKO 90, SKO 96 and Sabzaar oats

Table 3: Total phenolic content and antioxidant potential of different fractions of oats varieties

Varieties	Fractions	TPC *	DPPH * (IC ₅₀)	ABTS * (IC ₅₀)	NO * (IC ₅₀)
SKO 90	HEX	293.8±24.5 ^a	- ^a	2.83±0.19 ^a	> 5 ^a
	EA	8549.3±356.2	0.28±0.04	0.30±0.02	0.81±0.03
	BUT	5653.6±300.6 ^a	> 5 ^a	0.53±0.05 ^a	> 5 ^a
	WAT	805.7±50.6 ^a	> 5 ^a	3.04±0.16 ^a	> 5 ^a
SABZAAR	HEX	456.4±106.4 ^a	- ^a	2.7±0.45 ^a	> 5 ^a
	EA	6752.9±345.1 ^b	2.35±0.48 ^b	0.35±0.01 ^b	2.47±0.4 ^b
	BUT	2772.4±311.6 ^a	> 5 ^a	0.95±0.05 ^a	> 5 ^a
	WAT	685.0±92.7 ^a	> 5 ^a	2.44±0.26 ^a	> 5 ^a
SKO 96	HEX	976.1±51.1 ^a	- ^a	2.41±0.39 ^a	> 5 ^a
	EA	6835.6±805.3 ^b	1.73±0.55 ^b	0.29±0.02 ^{ns}	2.31±0.5 ^b
	BUT	4043.0±338.1 ^a	> 5 ^a	0.58±0.04 ^a	> 5 ^a
	WAT	1162.3±89.1 ^a	> 5 ^a	1.61±0.44 ^a	> 5 ^a

*All the values are expressed as means ± SD (n = 3)

Abbreviations: Total Phenolic Content (TPC), Gallic acid (GA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and Nitric Oxide scavenging (NOS), 50 % Inhibitory concentration (IC₅₀), HEX (Hexane), EA (Ethyl acetate), BUT (n-Butanol) and WAT (water).

^ap<0.05 as compared with EA fraction and ^bp<0.05 as compared with SKO 90 variety and ns: not significant as compared with SKO 90 variety according to one way analysis of variance and post hoc Dunnet's test

As expected, the radical scavenging activities of water and n-hexane fractions were the weakest as shown in Table 3. Thus the antioxidant compounds are mainly enriched in the EA-soluble fraction, significantly higher than that of other fractions at the same concentrations. This is in agreement with the reported literature (Cai *et al.*, 2011). It was also clear that the IC₅₀ of EA fraction was the lowest among the different investigated fractions (p<0.05). The IC₅₀ of SKO 90 (0.28±0.04 mg/ml) variety was significantly (p<0.05) lower than other varieties. ABTS assay also showed enrichment of phenolics in the ethyl acetate-soluble fraction of SKO90 and SKO96 studied varieties. However, there was no significant (p>0.05) difference in ABTS scavenging activities among EA fraction of all three varieties of oats. Like DPPH assay, the nitric oxide scavenging assay also demonstrated significantly (p<0.05) low IC₅₀ values for ethyl acetate-soluble fraction of SKO 90 (0.81±0.03 mg/ml) compared to other varieties.

These results suggest that ethyl acetate-soluble fraction of SKO 90 oats variety has comparatively higher phenolic content, which can be correlated to its lower IC₅₀ values in DPPH and NO assays.

CONCLUSION

A comparative study was carried out in the present work to ascertain maximum antioxidant potency among three Indian oats varieties (SKO 90, SKO 96 and Sabzaar). Based on total phenolic content and three *in vitro* antioxidant assays and it can be concluded that SKO 90 variety possesses highest total phenolic content and antioxidant activity. Looking at the several biological activities of natural antioxidants, the enriched ethyl acetate fraction of SKO 90 oats has the potential to be used as a dietary supplement. Further *in vivo* studies are warranted to prove the potential health benefits of ethyl acetate extract of SKO 90 oats.

MATERIALS AND METHODS

Chemicals and reagents

Studied three oats varieties SKO-90, Sabzaar, SKO-96 were procured from Sher-e-Kashmir University of Agricultural Sciences and Technology (SKUAST), Srinagar. Gallic acid, Folin Ciocalteu reagent, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6- sulfonic acid (ABTS⁺) diammonium salt, potassium persulfate, sodium nitroprusside, sulphanilamide, N-(1-naphthylethylenediamine) orthophosphoric acid, dimethyl sulphoxide (DMSO) were obtained from Sigma. Absolute ethanol (99.9%) was obtained from Changshu Yangyuan chemicals, China. All other reagents and chemicals used in the study were of analytical grade unless otherwise specified.

Preparation of fractions from three varieties of oats

The different solvent fractions were prepared from three oats varieties according to the reported method with some modifications (Cai *et al.*, 2011). The oats grains were cleaned manually to remove all foreign matter such as dust, dirt, stones and chaff. Whole grain oats were ground in a blender until the powder passed through a 0.5 mm sieve. About 10 g of each variety (in triplicate) was ultrasonically extracted with 100 ml of ethanol/water/acetic acid (80:20:0.1, v/v/v) at 30 °C for 30 min. The mixture was then extracted in a shaking water bath (300 rpm) at 60 °C for 2 h. After cooling to room temperature, the slurries were centrifuged at 4000 g for 15 min. The residue was further extracted with the same mixed solvent under the same conditions. The supernatants were pooled and filtered through Whatman filter paper No. 41. The solution was then evaporated under reduced pressure at 55 °C to obtain the crude extract and weighed. The ethanol extract was sequentially extracted with n-hexane, ethyl acetate and n-butanol, using liquid–liquid partition with water. After removal of the solvents, four fractions were obtained, namely n-hexane (HEX), ethyl acetate (EA), n-butanol (BUT) and water (WAT) fractions for each variety of oats. Total phenolic content and radical-scavenging activity of all the solvent fractions of each variety were evaluated.

Gas Chromatography analysis of hexane extracts of oats varieties

The fatty acid composition of all three varieties of oats was determined by gas chromatography (GC) after methylation by treating lipids in hexane fraction (0.5 ml) with 5 ml of 2 % sulphuric acid in methanol at 65 °C for 6 hrs in oil bath. Fatty acid methyl esters were then extracted using ethyl acetate and water and analyzed on GC equipped with the split injector and flame ionization detector. The injector and detector temperature were maintained at 230 and 250 °C respectively and the split ratio was 1:10. Separations

were performed on DB 225 capillary column (30 m x 0.25 mm id x 0.2 mm film thickness). The initial column temperature was 160 °C for 2 min, then rose to 230 °C at 5 °C min⁻¹ and finally kept at temperature of 230 °C for 20 min. Nitrogen, at a flow rate of 1.5 ml min⁻¹ was used as carrier gas. Peaks were detected at 250 °C, recorded and integrated using the software HP Chemstation and were identified by comparing their retention times with those of standards. Peak areas were calculated and percentages of fatty acids were obtained as weight % by direct normalization.

Determination of total phenolics

Total phenolic content in the various fractions of three varieties of oats were estimated by the Folin–Ciocalteu method as gallic acid equivalents (GAE) and expressed as milligrams of gallic acid per 100 gram of fraction (Singleton, Orthofer, and Lamuela-Raventós, 1998). Briefly, 25 µl of extract (5 mg/ml in DMSO) was mixed with 250 µl of Folin–Ciocalteu reagent (2N) followed by the addition of 2.5 ml of distilled water. After 1 min of incubation at room temperature, 250 µl of Na₂CO₃ solution (20% aqueous solution) was added to the mixture and incubated at room temperature for 1 h. The absorbance was measured at 765 nm and the total phenolic content was calculated as GAE from a calibration curve (gallic acid) and expressed as mg/100 g of the extract. The data were presented as the average of triplicate analyses.

Determination of in vitro free radical scavenging activity

DPPH Assay: The DPPH free radical scavenging activity of different fractions of oats extract was determined in triplicate according to the reported method (D. Peterson, Emmons, and Hibbs, 2001). Briefly, 100 µl of Tris buffer (0.1 M, pH 7.2) and 125 µl of 0.5 mM methanolic DPPH solution was added to 25 µl of the extract (5 mg/ml in DMSO). The mixture was incubated at room temperature for 30 min in dark and absorbance was measured at 517 nm. The DPPH free radical scavenging activity was expressed by IC₅₀ value, which is the concentration of sample required to decrease the absorbance at 517 nm by 50%.

ABTS Assay: The ABTS free radical scavenging activity of different fractions of oats extract was determined in triplicate according to the reported method (Re, Pellegrini, Proteggente, and Pannala, 1999). Briefly, ABTS⁺ radical cation was generated by a reaction of 7 mM ABTS with 2.45 mM potassium persulfate. The reaction mixture was allowed to stand in the dark for 16 h at room temperature and used within two days. The ABTS⁺ solution was diluted with ethanol, to give an absorbance of 0.70 ± 0.050 at 734 nm. To a volume of 10 µl of the extract (5 mg/ml in DMSO), 190 µl of ABTS radical solution was added. The mixture was incubated at room temperature for 15 min in dark and absorbance was measured at 734 nm. The ABTS⁺

scavenging rate was calculated to express the antioxidant ability of the sample. IC₅₀ values calculated denote the concentration of sample required to decrease the absorbance at 734 nm by 50%.

Nitric Oxide Scavenging Assay: The nitric oxide scavenging ability of different fractions of oats extract was determined in triplicate by Griess reaction using a reported method (Ahmad and Azam, 2011). Briefly, sodium nitroprusside (100 µl, 10 mM) was added to 30 µl of oats extract (5 mg/ml in DMSO) and incubated at room temperature for one hour. Then 50 µl of sulphanilamide (1% in 5% orthophosphoric acid) was added and incubated at room temperature for 10 min followed by the addition of 50 µl of 0.1% (Naphthyl)ethylenediamine (NED). The contents were shaken well before measuring absorbance at 540 nm. IC₅₀ values calculated denote the concentration of sample required to decrease the absorbance at 540 nm by 50%.

Statistical analysis

All statistical analyses were performed using one way ANOVA with the Graph Pad Prism, version 5.0 software. Comparisons between varieties were performed by applying Dunnett's multiple comparison procedure with reference to SKO90 variety. And comparison between fractions were made by applying Dunnett's post-hoc test with reference to ethyl acetate fraction. Results were expressed as Means ± S.D. Statistical significance was considered at p<0.05.

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CONFLICTS OF INTEREST

The authors have declared no conflict of interest.

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