DPPH Radical Scavenging Assay, Biological Activities, Nutritional Composition and Quality Parameters of *Momordica charantia* Seeds Grown in District Charsadda, KPK, Pakistan

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Abstract. The nutritional composition, physicochemical characteristics, antimicrobial activity and DPPH free radical scavenging activity of the oil extracted and proximate and elemental composition of seed cake of the local variety of *Momordica charantia* were examined as per AOAC or elsewhere mentioned procedures. Physicochemical properties of the oil exhibited colour (1.71 R/U, 1.19 Y/U) being reddish brown, the higher iodine value (105.5), saponification value (190.7), low acid value (1.3) and the higher proportion of unsaturated fatty acids (especially the omega-3 fatty acid) as compared to saturated fatty acids of the oil are an indication of its edibility and industrial utility. The oil also showed good inhibitory action against the selected fungal and bacterial strains as well as DPPH free radical scavenging activity. Results of the proximate composition of the seed cake showed it to be good source of total proteins (18. 17%), crude fibre 10.37%, percent oil (38.1%) and elements such as calcium (374.9 mg/100 g), copper (3.01 mg/100 g), iron (39.57 mg/100 g) and zinc (12.04 mg/100 g). It can be concluded from the study that oil and seed cake of the local variety of bitter gourd seed (BGS) oil possesses a good nutritional, antioxidant and antimicrobial potential.

Keywords: Momordica charantia, bitter gourd seed, physicochemical properties, fatty acid, antimicrobial potential

Introduction

Momordica charantia belonging to the Curcurbitaceae family and commonly known as bitter gourd, is a vegetable crop cultivated in humid subtropical regions of the globe (Lucas *et al.*, 2010). It is a commonly grown vegetable in Pakistan and is being used as a cure in diabetes mellitus and other therapeutic evidences. Bitter ground is an effective antidiabetic, anticancer, antimicrobial, anti- hepatotoxic, antiviral, antiulcerogenic having an antioxidant and larvicidal properties (Tuan *et al.*, 2011).

Different parts of the plant are used and the seed is found being a rich source of oil (18.1-37.6%) and protein (28-30%). The protein of the seed has been found to be a good source of essential amino acids which can be utilized as a good functional ingredient in food systems. The seed also supply important dietary minerals such as P, K, MG, S, and Ca, along with a variety of flavonoids, phenolic compounds and other antioxidants (Horax *et al.*, 2010). *Momordica charantia* seed oil possesses many essential nutrient compounds needed for proper functioning of human body. The oil extracted from the seed contain appreciable amount of conjugated linoleic acid and á-eleostearic acid which may be of help in reducing the risk of coronary heart diseases in non-diabetic as well as diabetic patients. It not only inhibits pathogenesis and replication of psoriasis but leukemia and cancer as well. The oil also has a utility in paint industries (Ahmad and Khan, 2013).

The present study aimed to assess the physicochemical properties, antioxidant and antimicrobial potential of the BGS oil and nutritional composition of bitter gourd seed cake/flour from the BG (bitter gourd) cultivar commonly grown in district Charsadda, KPK, Pakistan.

Materials and Methods

Procurement of bitter gourd seeds (BGS). Ripened fruits of a local variety (district Charsadda) were purchased from the local vegetable market. The seeds were separated and were washed several times with

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water to remove any plant and foreign material. Later the seeds were dried in an electric oven until constant weight was achieved. The dried seeds were stored in airtight jars in triplicates for further analyses.

Oil extraction. Dried seeds were grinded in an electric grinder. The ground seed powder was soaked in *n*-hexane for 24 h. After soaking for a day the extract was filtered through filter paper and was then concentrated /distilled in rotary evaporator under reduced pressure at 40-45 °C. Percent oil was calculated and the oil and cake were directly used for quality parameters.

Proximate composition and mineral content determination. After oil extraction the seed cake /residue/flour samples were tested for proximate and elemental composition. By using AOAC official methods (1990) total protein content of the seed cake was estimated by a micro Kjedlhal apparatus. Crude oil was estimated by Soxhlet method while crude fibre and ash contents were determined by the AOAC official methods (1984). For minerals analysis samples were first subjected to acid digestion and later were analysed by atomic absorption spectrophotometer (AAS) methods (AOAC, 2005).

Oil analysis. The physical and chemical parameters of the crude oil were determined as per AOAC official methods (AOAC, 2005). Refractive index, density, specific gravity, surface tension, acid value, free fatty acid value, saponification value, iodine value, colour and peroxide value were carried out.

Fatty acids analysis. Preparation of FAMES. About 25-40 mg of oil sample were weighed in FAMEs tubes and 1.5 mL methanolic sodium hydroxide (0.5 N) was added. The tubes were stoppered with screw capes. The mixture was heated in a boiling water bath for 05 min. Tubes were cooled to room temperature and 2.5 mL BF₃ (10 % in MeOH) was added. Tubes were again heated in boiling water for half an hour. Cooled at room temperature and 5 mL brine solution + 1 mL n-hexane was added. The tubes were shaken vigorously on vortex and then the layers were allowed to separate. The upper (hexane) layer was taken through pasture pipette. For GCMS analysis about 1 mL n-hexane was added again to repeat the above steps to separate the hexane layer. The volume was adjusted to 2 mL, filtered through 45 µm membrane filter and transferred to GC vial for injection into GC-MS.

Identification of fatty acids by GC-MS. The methyl esters of the oil were analysed for the respective fatty acid composition by gas chromatography mass spectrometry. The equipment used for this purpose was Shimadzu GC-MS- QP 2010 plus using a capillary column TRB FFAP (30 m x 0.25 mm i.d). The temperature programming of the column oven was set as 50 – 220 °C with rise of 5 °C/ min. Helium was used as the carrier gas and its total flow was adjusted to 77.1 mL/min while column flow was 3.29 mL/min at split ratio of 20.0. The temperatures of injector, interface and ion source were set at 240, 240 and 250 °C, respectively. The peaks were identified by comparison of their retention time with those of the standard methyl esters (FAMEs standard mix, 37 components, Sigma Aldrich) analysed under the same conditions.

Antimicrobial assay. The extracted oil of BG was tested against different strains for anti-infective potential. Antibacterial activity against three gram negative bacteria (Xnythomonas campestris, Proteus mirabilis, Escherichia coli) and one gram positive bacteria(Bacillus cereus) and four pathogenic fungi namely Phythium ultimum, Paecilomyces lilacinus, Rhizopus stolonifer and Tricoderma harzanium were tested. The pure microbial strains were obtained from Agriculture Chemistry Lab, Department of Agricultural Chemistry, KPK Agriculture University Peshawar. Agar well diffusion was used for fungi and streaking method was used for bacteria. The minimal bactericidal (MBC) and fungicidal (MFC) potential as zones of inhibition (mm) were determined at different concentrations (0.2, 0.4, 0.5, 0.6, 0.8 mg/µg) of the stock solutions of BGS crude oil in sterile DMSO. Standard drug Amoxicillin and Miconazole were used as positive control while sterile DMSO was used as negative control for the purpose of comparison as per procedure of Sheikh et al. (1995).

Antioxidant activity by DPPH free radical scavenging methods. DPPH radical scavenging activity was performed by following the method of Rauf *et al.* (2012). About 25 mg of oil was dissolved in distilled methanol and was diluted up to 50 mL. From the stock solution 10, 20,40,60,80,100 mg/mL dilutions were prepared. Of each solution 5 mL was taken in a test tube to which 1 mL of 0.01 M of DPPH solution was added. They were kept in dark for test minutes along with a control (5 methanol+ 1 mL DPPH solution). After the incubation for 30 min the antioxidant activity was measured by

using Optima UV- visible spectrophotometer at wave length of 517 nm. The experiments were performed in three replicates. Quercetin was taken as a standard for the purpose of comparison. Percent radical scavenging activity was calculated as:

% DPPH = control absorbance- extract absorbance \times 100/control absorbance

Statistical analysis. Data collected from the triplicate analyses was analysed for means, standard deviations and one way ANOVA at P=< 0.05 level of significance wherever needed.

Results and Discussion

Proximate and elemental composition. Results of the proximate and elemental composition of the BG seed cake/flour are given in Table 1. The data showed seed cake contained percent moisture 7.43%, crude protein 18.17%, crude fibre 10.37%, ash 4.48% and total carbohydrates 28.88%, respectively. In contrast to present study Anjum *et al.* (2013) and Prasantha *et al.* (2009) reported much higher percent moisture (22.9-29.32% and 53-79.5%), respectively, whereas Ali *et al.* (2008) and Nayam *et al.* (2009) recorded moisture level similar to the present study (7.62, 8.20% 7.3%), respectively. These differences may be attributed to genetic or maturity variations of the collected samples. The oil content (given as a percentage to the dry weight) of the sample was 38.1% which is higher than the values

 Table 1. Proximate and elemental composition of bitter
 gourd seed

Parameter	Content*	P-Level**
Proximate values		
Moisture (w/w %)	7.43±0.23	1.50^{a}
Percent oil (w/w %)	38.1±3.03	0.07^{a}
Crude protein %	18.17±6.09	0.06^{a}
Crude fibre %	10.37±0.26	0.06^{a}
Ash %	4.48±0.52	0.08^{a}
Carbohydrates %	28.88±2.37	0.14 ^a
Minerals (mg/100 mg)		
Calcium	374.21±7.99	0.04^{a}
Iron	3.01±0.08	0.64^{a}
Copper	39.57±3.84	0.72^{a}
Zinc	12.04±6.87	0.07^{a}

* = values represent means of three replications; ** = numbers carrying same alphabets exhibit non significance at 0.05 levels.

reported by Ali et al. (2008) and Nyam et al. (2009) being 19.30% and 26.00%, respectively. However, the oil content was near to the findings of 41-45% and 31.51-40.79% by Islam et al. (2011) and Anjum et al. (2013), respectively. Umamaheshwari and Naik (2013) reported 35% oil in BGS. The mean of total protein content of the samples was found to be 18.17% indicating BGS as a rich source of protein. This amount (18.17%) of the present study was in agreement with the finding of Yuwai et al. (1991) Ali et al. (2008) and Anjum et al. (2013) being 18.02%, 18.23-23.36%, and 14.92-19.17%, respectively though Nyam et al. (2009) reported lower protein content (11.8%). Total carbohydrates as estimated were 28.88% which are in agreement with the findings of Anjum et al. (2013), Ali et al. (2008), and Nyam et al. (2009). BG seed cake contained appreciable amount of calcium (374.21 mg/100 g) and copper (3.01 mg/100 g), iron (39.57 mg/100 g) and zinc (12.04 mg/100 g) indicating BGS as a good source of minerals also. Similar results were also reported by Ali et al. (2008), Anjum et al. (2013), and Bakare et al. (2010) while iron and zinc content found in the current study are slightly higher than those studied by Aussubaie and EL-Garawany (2004).

Physicochemical properties of BGS oil. Results of different quality parameter of the oil are presented in Table 2. A slight variation in the density (1.33 kg/mg) was found from the findings of Anjum et al. (2013), Ali et al. (2008) and Assubaie and El-Garawary (2004) being 0.9709-0.9978, 0.9962 and 0.9448-0.9605 mg/mL at 25 °C, respectively. The refractive index of the oil (1.4995 at 25 °C) is closely related to the values reported as 1.4888, 1.485, 1.4895 and 1.480 by Ali et al. (2008); Umamaheshwari and Naik (2013); Prasantha et al. (2009) and Anjum et al. (2013), respectively. The specific gravity of the oil samples was 0.87. The colour of BGS oil was 1.71 red units (1 inch/cell) while it was 1.2 yellow unit (1 inch/cell) indicating the presence of polar substances which can work as anti-oxidative agents. The surface tension (2.33), viscosity (7.0 at 21 °C) kinematic viscosity (38.32) lower acid value (1.3 mg of KOH/g), the high saponification value (190.7 mg of KOH/g) and iodine value (105.5 g of $I_2/100$ g) indicated the potential uses of the oil in diet and industries. The saponificaion value of the present findings are in agreement with those of Ali et al. (2008) and Prasantha et al. (2009) as 187.0 and as 187.01, respectively. Anjum et al. (2013) also reported iodine value as 184.02-186.09. The percent free fatty acid of

0.7 g/100 g are similar to the findings of 0.86 and 1.06 as reported by Ali *et al.* (2008), Umamaheshwari and Naik (2013) and Anjum *et al.* (2013). Peroxide value

 Table 2. Physicochemical characteristics of the BGS oil

Parameters	Content*	P- level
Colour (red units)	1.71±0.24	2.7 ^{a**}
Colour (yellow units)	1.2±0.81	0.90^{a}
Refractive index at 25 °C	1.4995 ± 0.04	0.07^{a}
Density (20 °C kg/m ³)	1.33 ± 0.02	0.09 ^a
Specific gravity	0.87 ± 0.08	0.53 ^a
Surface tension	2.33 ± 0.081	0.07^{a}
Viscosity (21 °C)	70±0.47	0.3 ^a
Kinematic viscosity (mm ² /sec)	$38.32{\pm}0.93$	0.28^{a}
Acid value (mg KOH/g)	1.3±0.21	0.36 ^a
Free fatty acid (g/100 g)	0.7±0.15	0.14 ^a
Saponification value		
(mg KOH/g of oil)	190.7±0.5	0.93 ^a
Iodine value (g of $I_2 / 100$ g)	105.5±3.3	0.91 ^a
Peroxide value	3.1±0.7	0.04^{a}
(meq of O ₂ /kg of oil)		

* = values are means of three replicates; ** = numbers carrying same alphabets exhibit non significance at 0.05 levels.

Fatty acids and percent saturated vs unsaturated fatty acids distribution of the BGS oil. Fatty acid composition of BGS oil is presented in Table 3 and Fig. 1. BGS oil contained appreciable amounts of stearic acid (66.57%), oleic acid (9.76%) followed by palmitic acid (0.85%), linolenic acid (2.19%), arachidic acid and behenic acid (1.04% and 1.93%), respectively. Similar stearic acid values were reported from the oil of Chinese BGS oil; however, Anjum et al. (2013) reported 29.14% stearic acid. Ali et al. (2008) also reported lower values for stearic acid, higher values for oleic acid and similar values for palmitic acid. The drastic variation in the fatty acid composition may be attributed to variation in the soil condition, type of variety, and climatic conditions in which the tested BG were grown.

DPPH free radical scavenging potential of BGS oil. DPPH free radical scavenging activity of BGS oil is shown in Fig. 2. Observed scavenging activity (mg/mL DPPH scavenging) demonstrated by BGS oil as 23.89,

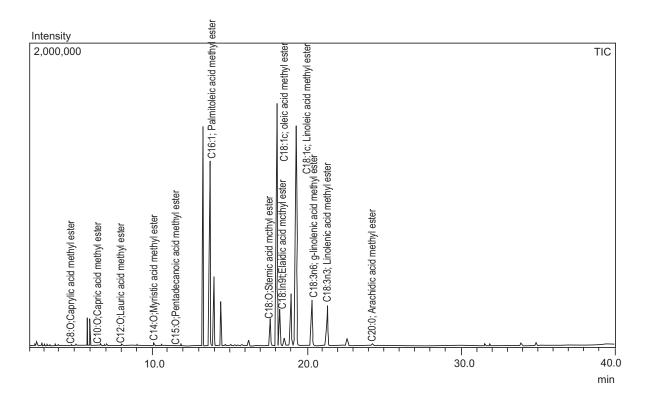


Fig. 1. Chromatogram of BGS oil fatty acids methyl esters.

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Fatty acids	%age of concentrations*
Caprylic acid C _{8:0}	$0.098{\pm}0.03$
Capric acid C _{10:0}	0.05 ± 0.02
Lauric acid $C_{12:0}$	0.05±0.015
Myristic acid C _{14:0}	$0.185{\pm}0.52$
Palmitic acid C _{16:0}	4.72±0.83
Palmitoleic acid C _{16:1}	0.85 ± 0.65
Stearic acid C _{18:0}	66.57±0.29
Oleic acid C _{18:1}	9.76±0.03
Lenoleic acid C ₁₈ : ₂	$0.24{\pm}0.05$
<i>q</i> -Linolenic acid C ₁₈ : _{3n6}	1.91 ± 0.92
Linolenic acid C _{18:3n6}	2.19±0.06

 1.04 ± 0.87

1.93±0.09 ND

74.43±0.02

 14.94 ± 0.08

14.54±0.04

 2.97 ± 0.06

Table 3. Fatty acids composition of the BGS oil

* = means of triplicate analysis.

Arachidic acid C₂₀:0

Lignoceric acid C24:0

Total unsaturated acid

Total saturated acid

18/16 ratio

20-24 ratio

Behenic acid C22:0

22.16, 20.50, 19.75, 17.86, and 11.96% at the tested doses of 100, 80, 60,40, 20 and 10 ppm, respectively as against 100% scavenging by the standard quercetin. It was observed that the extracted oil exhibited overall low antioxidant activity as compared to fruit matter or dry matter (135-144%) being reported by Anjum *et al.*

(2013); Ahmad *et al.* (2014) and Ozusaglam and Kara koca (2013). However it can be assumed from the results that BGS oil possesses some antioxidant activity due to the oil will have a longer shelf life and it can be utilized in the preparation of value added food based products.

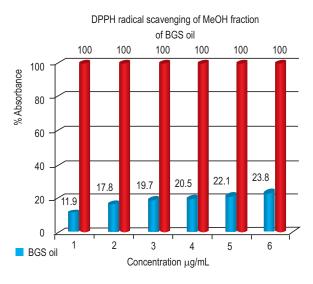


Fig. 2. Antioxidant DPPH free radical scavenging potential of BGS oil.

Table 4. Summary o	of the antibacteria	al activity of BGS oi	il
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Concentration (mg/ μ L)	Zone of inhibition (mm)*			
	Escherichia coli	Bacillus subtilis	Proteus mirabilis	Xynthomonas campestris
0.2	0.6 ± 0.40	1.2 ± 0.67	0.08 ± 0.09	0.53 ± 0.37
0.4	2.1 ± 0.08	2.2 ± 1.02	0.16 ± 0.05	2.03 ± 0.05
0.5	4.43 ± 0.37	2.86 ± 1.34	0.53 ± 0.29	3.26 ± 0.20
0.6	5.96 ± 0.05	4.3 ± 0.75	1.13 ± 0.26	3.8 ± 0.62
0.8	7.6 ± 2.29	6.96 ± 0.05	1.46 ± 0.37	7 ± 0.81
Standard amoxicillin	10.36 ± 0.44	8 ± 0.81	6.46 ± 0.37	10 ± 0.08
DMSO	0	0	0	0

* = values are in terms of mean±SD of results done in triplicates.

Table 5. Sum	mary of antif	fungal activities	of BGS oil

Concentration (mg/ μ L)	Zone of growth (mm)			
	Paecilomyces lilacinus	Phytium ultimum	Rhizopus stolonifer	Tricoderma harzianum
0.2	17.8 ± 0.25	18.96 ± 0.78	21.06 ± 0.75	24.9 ± 0.53
0.4	12.5 ± 0.41	16.5 ± 0.08	20.7 ± 0.08	23.1 ± 0.94
0.5	10.6 ± 0.08	15.9 ± 0.05	20 ± 0.006	20.86 ± 0.65
0.6	9.5 ± 0.08	14.3 ± 0.07	18 ± 0.006	15.7 ± 0.26
0.8	8.5 ± 0.08	9.6 ± 0.08	17.16 ± 0.12	12.46 ± 0.37
Miconazole	19.3 ± 0.82	25.8 ± 0.16	22.7 ± 0.88	26.6 ± 0.43
DMSO	0	0	0	0

Antimicrobial assays of the BGS oil. The antibacterial and antifungal activities of Momordica charantia seed (BGS) oil against selected bacterial and fungal strains are presented in Tables 4-5. The data showed Escherichia coli and Xynthomonas campestris being the most sensitive bacterial strains against BGS oil as against the positive standard amoxicillin. Results of Escherichia coli are in agreement with the finding of Grover and Yadav (2004); Paul and Raychaudri (2010); Behara et al. (2010), Ahmad et al. (2014) and Sharma et al. (2011) showing the inhibitory ability of BGS oil for E. coli in the range of 2-12 mm. Similarly, antibacterial activity against Bacillus subtilis was according to the findings of Sharma et al. (2001) and Fritsch et al. (2007). The zone of inhibition against Proteus mirabilis and Xanthomonas campestris were similar to the finding of Leelaprakash (2011); Ahmad et al. (2014) and Ozusaglam and Karakoca (2013), respectively. Results of the antifungal activity of GBS against fungal strains showed the trend as follows:

Tricoderma harzianum > Rhizopus stolonifer > Phytium ultimum > Paecilomyces lilacinus.

These results obtained for antifungal potential as the zone of growth of BGS oil were similar to the findings of Ahmad and Khan (2013) and Ozusaglam and Karakoca (2013) against *Rhizopus* and *Trichoderma*. Inhibitory activity for *Paecilomyces* and *Pythium* were in accordance with the finding of Anjum *et al.* (2013) and Ahmad *et al.* (2014). It can be concluded from the present findings that bitter melon seed oil possesses a very good antimicrobial potential against pathogenic bacteria and the studied fungal strains.

Conclusion

It can be concluded from the findings of the current study that BGS oil is a good source of dietary lipids due to its higher level of unsaturated fatty acids in seed oil and protein content in the seed cake. Due to its higher iodine value, low peroxide and free fatty acids properties it can be considered as a new and valuable source of edible oil having prolonged shelf life. The oil can have industrial utility in the manufacturing of margarine, soap production, and in paints and coating industry. Due to its good antimicrobial properties the oil can be used as antibacterial and antifungal agents in pharmaceutical preparations. It can also be utilized for blending with other conventional edible oils and in the preparation of nutraceuticals for health promotion.

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