Study of Quality Characteristics and Efficacy of Extraction Solvent/Technique on the Antioxidant Activity of Bitter Gourd Seed

Fozia Anjum1*, Muhammad Shahid2, Shazia Anwer Bukhari1, Shakeel Anwar3 and Sajid Latif4

1Department of Chemistry, Government College University, Faisalabad, 3800, Pakistan
2Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, 38040, Pakistan
3Department Plant Pathology, University of Agriculture, Faisalabad 38040, Pakistan
4Institute for Animal Production in the Tropics and Subtropics (480B), University of Hohenheim, 70593 Stuttgart, Germany

Abstract

Bitter Gourd Seed (BGS) oil of two varieties were evaluated for physicochemical composition, antimicrobial and hemolytic activities however BGS residue was appraised for proximate composition, mineral contents and the efficacy of extracting solvents; ethanol, 80% ethanol and water; extraction techniques; shaking, reflux and ultrasound on antioxidant activity of BGS extract was evaluated. Significantly (P<0.05) higher oil and protein content was observed in the BGS-1 (40.8 and 19.2% respectively) as compared to BGS-2 (31.5 and 14.9% respectively). Significant differences (P<0.05) were observed between their physicochemical parameters and oxidative stability. BGS oils of both varieties were rich in oleic acid, followed by stearic, and oleic acids. The concentration of α-lipoic acid was detected significantly (P<0.05) higher in BGS-1 oil as against the BGS-2 oil. A higher amount of antioxidant extract yield, flavonoid contents, phenolic contents, metal chelating and free radical scavenging activity were observed in the 80% ethanolic extract of both BGS under ultrasound treatment. Furthermore, BGS-1 showed remarkably higher level of antioxidants as compared to BGS-2. The seed oil of both varieties showed noteworthy antimicrobial activity against battery of selected bacteria and fungi strains, assessed by disc diffusion and measurement of minimum inhibitory by micro dilution method. Negligible hemolytic activity was recorded against human and bovine erythrocytes by BGS-1 variety.

Keywords: Momordica Charantia; Oxidative stability; Antioxidant; Antimicrobial and hemolytic activities; Reflux; Ultrasound

Introduction

Momordica charantia Linn belongs to the Cucurbitaceae family and commonly known as Bitter Gourd (BG), bitter melon, karela and grows in the humid and subtropical regions of the world. It is inherent in Asia and now widely cultivated in all parts of the world due to its dietary assessment in its immature or ripened fruits. The BG plant is modified to a wide variation of climates however its best production is in warm areas [1]. It is a curative vegetable which has been used conventionally for diabetes treatment. It is a ground-breaking plant for its adaptability to a wide variation of climates however its best production is in warm areas. It is a curative vegetable which has been used conventionally for diabetes treatment. It is a ground-breaking plant for its adaptability to a wide variation of climates however its best production is in warm areas.

Although the different parts of plant BG have been used as food and drug but the fruit is the most important part [3]. Depending on the maturity stages, BG Seed (BGS) has been found as a rich source of oil (18.1-37.6%) and protein (28-30%). BGS oil is rich in fatty acids like Conjugated Linoleic Acid (CLA). BGS protein is a good source of essential amino acids (Met, Cys, Ile, Phe, Tyr and Lys) and could be a good source of protein for functional ingredients in a food system. BGS also supply important minerals (P, K, Mg, S and Ca), flavonoids and phenolic compounds (catechin and gallic acid) [4]. Antioxidants, especially, the phenolics have gained considerable importance due to their potential health benefits. Consumption of plant foods containing antioxidants is beneficial to health as it lowers down many degenerative processes. Antioxidant compounds can be recovered from plant materials through different extraction techniques taking into account their chemistry and uneven distribution in the plant matrix. Antioxidant compounds most frequently isolated by using solvent extraction technique. However, the extract yields and resulting antioxidant activities of the plant materials are strongly dependent on the nature of extracting solvent, due to the presence of different antioxidant compounds of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent. Polar solvents are frequently employed for the recovery of polyphenols from a plant matrix. The most suitable of these solvents are (hot or cold) aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate [1].

Therefore huge effort has been made to develop effective selective extraction techniques.

The present study therefore has been conducted with the main objective of investigating the physicochemical composition, antimicrobial and haemolytic activity of BGS oil and to investigate the most effective solvent/technique for extracting potent antioxidant compounds from BGS. Proximate and nutritional compositions of BGS have been reported by few scientist but there is an information gap regarding chemical composition, antioxidant, antimicrobial and hemolytic activities of BG cultivated in Pakistan. This information can add value to the agricultural potential of BG, and can also introduce high-value components in the market towards a nutraceutical potential.

*Corresponding author: Fozia Anjum, Department of Chemistry, Government College University, Faisalabad, 3800, Pakistan, Fax: + 92-41-9200764; E-mail: fozianjaun2008@yahoo.com

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Materials and Methods

Bitter gourd seed (BGS)

Ripened fruits of two BG varities were procured from Ayub Agriculture Research Institute, Department of Oil Seed Crops, Faisalabad, Pakistan. These two varieties have different morphology from each other. The local variety BGS-1 (Guti) has small fruit, deep green color, covered with small triangular tubercles and strongly bitter in taste whereas another varietyity BGS-2 (Goj) has long green fruit, covered with long triangular tubercles and bitter in taste. In Pakistan, BG is commonly known as kerela. The seeds were separated from the fruits and washed several times with water to remove any dust particles and foreign materials. Afterward, the seeds were placed in Pyrex Petri plates (15.00 g in each Petri plate) to dry in an electric oven at 45°C for 24 h until a constant weight was attained. Seeds were stored in polyethylene bags till for further analysis. Duplicate samples of seeds were collected.

Reagents and standards

All chemicals of commercial as well as analytical grade used in this study were purchased from either E. Merck (Darmstadt. Germany) or Sigma-Aldrich (Buchs, Switzerland) unless otherwise noted. Pure standards of tocopherols [DL-α-tocopherol, (+)-δ-tocopherol, and (+)-γ-tocopherol], FAME, Catechin, Triton-X-100 and Gallic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO), microbial media and sensitivity disc for antimicrobial activity were from Oxoid (Hampshire, UK).

Oil extraction

After drying samples in an electric oven, thoroughly mixed seeds were ground by using an electric grinder. Each batch (~75-80.00g) of BGS was fed into Soxhlet extractor fitted with 500 mL round bottom flask and a condenser. The extraction was carried out on water bath (75-80°C) for 8-9 h using 300 mL of n-hexane. After extraction, the solvent was distilled off under vacuum in a rotary evaporator (Eyela Rotary Vacuum Evaporator N.N. Series) at 45°C and the percentage of the oil contents were calculated [5].

Analysis of BGS residue and mineral content determination

After oil extraction, residues contain protein, starch, carbohydrates and different inorganic minerals. Total protein contents of BGS residue were estimated according to AOAC official method [6] by using a micro-Kjeldhal apparatus whereas water soluble protein was analyzed by Lowry method using bovine serum albumin as the standard [7]. Starch contents and total carbohydrate were also estimated by modified method as reported by Ali et al [8]. Fiber and ash contents were analyzed according to the International Standard Organization (ISO) method [9].

For mineral analysis, BGS samples were subjected to acid digestion and analyzed by UV-Vis as well as by atomic absorption spectrophotometry (AAS) as described by AOAC, [10].

Analysis of BGS oil

Physical and chemical parameters: Determination of density, refractive index, FFA, PV, iodine value, ester value, saponification value, unsaponifiable matter, Reichert-Meissl Value and p-Anisidine value of the extracted oil was carried out according to standard IUPAC method. Color was estimated by Lovibond tintometer (Tintometer Ltd., Salisbury, United Kingdom) using a 1-in. (2.5 cm) cell. Specific extinction at 232 and 270 nm was determined using Hitachi U-2001 spectrophotometer by following the method of IUPAC [11]. Pour flash, fire and smoke point was analyzed according to the method of AOCS [12].

Tocopherol and fatty acid analysis: Tocopherol analysis was performed by HPLC following the method of Anjum et al. [13] whereas fatty acid composition was determined by instrumental methods like GLC. GLC analysis was performed by preparing fatty acid methyl ester (FAME) according to IUPAC standard method 2.301 [14] and analyzed on a Shimadzu gas chromatograph as reported by Anjum et al [13].

Antimicrobial activity: The extracted oils of both BGS varities were individually tested against a set of microorganisms, including two Gram-positive bacteria: Staphylococcus aureus (S. aureus), API Staph TAC 6736152, Bacillus subtilis (B. subtilis) JS 2004, two Gram-negative bacteria: Escherichia coli (E. coli) ATCC 25922, and Pasteurella multocida (P. multocida) (local isolate) and four pathogenic fungi, Candida albicans (C. albican), Microsporum canis (M. canis), Aspergillus flavus (A. flavus) and Fusarium solani (F. solani). The pure microbial strains were obtained from Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. Purity and identity were verified by the Department of Microbiology, University of Agriculture, Faisalabad, Pakistan. Bacterial strains were cultured overnight at 37°C in Nutrient agar (NA, Oxoid) while fungal strains were cultured overnight at 28°C using Potato dextrose agar (PDA, Oxoid).

Disc diffusion method: The antimicrobial activity of both BGS oils was determined by disc diffusion method. Briefly, 100 µL of suspension of tested microorganisms, containing 10° colony-forming units (CFU)/mL of bacteria cells and 10° spores/mL of fungi spread on NA and PDA medium, respectively. The filter discs (6 mm in diameter) were individually impregnated with 15 µL of extracted oil, placed on the agar plates which had previously been inoculated with the tested microorganisms. Discs without samples were used as a negative control. Amoxycillin (30 µg/dish) (Oxoid, UK) and Flumequine (30 µg/disk) (Oxoid, UK) were used as positive reference for bacteria and fungi, respectively to compare sensitivity of strain/isolate in analyzed microbial species. Plates, after 2 h at 4°C, were incubated at 37°C for 18 h for bacteria and at 28°C for 24 h for fungal strains. Antimicrobial activity was evaluated by measuring the diameter of the growth inhibition zones (zone reader) in millimeters for the organisms and comparing to the controls [15].

Determination of minimum inhibitory concentration (MIC): For the determination of MIC (which represents the concentration that completely inhibits the growth of microorganisms) a micro-dilution broth susceptibility assay was used, as recommended by National Committee for Clinical Laboratory Standards [15]. All tests were performed in nutrient broth (NB, Oxoid, UK) for bacterial and sabouraud dextrose broth (SDB, Oxoid, UK) for fungal strains supplemented with Tween 40 detergent to a final concentration of 0.5% (v/v). Bacterial strains were cultured overnight at 37°C in NB and the fungi were cultured overnight at 28°C in SDB. Dilutions series were prepared from 0.03 to 72.0 mg/mL of the compounds in a 96-well microtiter plate, 160 µL of NB and SDB for bacteria and fungi, respectively were added onto microplates and 20 µL of tested solution. Then, 20 µL of 5x10° CFU/mL of standard microorganism suspension were inoculated on to microplates. Plates were incubated at 37°C for 24 h for bacteria, and at 28°C for 48 h for fungi. The same test was performed simultaneously for the growth control (NB + TWEEN 40) and sterility control (NB + TWEEN 40 + test compound). Amoxycillin
was used as a reference compound for antibacterial and Flumequine for antifungal activities. The growth was indicated by the presence of a white "pellet" on the well bottom.

**Hemolytic activity:** Hemolytic activity of the oil was studied by the method used by Sharma and Sharma [16]. Three mL freshly obtained heparinized human blood was collected from volunteers after consent and counseling and bovine from the Department of Clinical Medicine and Surgery, University of Agriculture. Blood was centrifuged for 5 min at 1000xg plasma was discarded and cells were washed with three times with 5 mL of chilled (4°C) sterile isotonic Phosphate-buffered saline (PBS) pH 7.4. Erythrocytes were maintained 10^6 cells/mL for each assay. Hundred µL of each oils were mixed with human and bovine erythrocytes (10^6 cells/mL) separately. Samples were incubated for 35 min at 37°C and agitated after 10 min. Immediately after incubation the samples were placed on ice for 5 min then centrifuged for 5 min at 1000xg. Supernatant 100 µL was taken from each tube and diluted 10 time with chilled (4°C) PBS. Triton X-100 (0.1% v/v) was taken as positive control and phosphate buffer saline (PBS) was taken as negative control and pass through the same process. The absorbance was noted at 576 nm using µQuant (Bioteck, USA). The % RBCs lysis for each sample was calculated.

**Preparation of extracts for antioxidant activity:** BGS extract was prepared by using 100 g of BGS powder of both types with 1.0L of each of the solvent-water. Absolute ethanol and aqueous ethanol for 3 hours in an orbital shaker (Gallenkamp, UK), or under reflux on a water bath or in ultrasonic bath (1 hour) in separate experiments then filtered with filter paper (whatman filter paper). The residues were re-extracted twice. Extract was concentrated and lyophilized. The dried crude concentrated extracts were weighed to calculate the yield and stored at 4°C till for further analysis [17].

**Total phenol content (TPC):** The TPC of both BGS extracts were determined according to the Folin-Ciocalteu method described by Chaovanalikit and Wrolstad [18]. BGS extracts were diluted with 2.5 mL of distilled water, 0.5 mL of the Folin-Ciocalteu stock reagent and 1.0 mL of Na2CO3 reagent (75 g/l) were added to the mixture. The mixture was incubated for 30 min at 30°C and then the absorbance was measured at 765 nm. TPC was expressed in milligrams of Gallic acid equivalents per gram of BGS extract.

**DPPH radical scavenging activity:** DPPH radical scavenging activity was determined by following the modified method of Chang et al. [19]. 1.0 mL of extract containing 25µg/L of dry matter dissolving in methanol, 5.0 mL of freshly prepared solution of 1, 10-diphenyl-2-picrylhydrazyl (DPPH) at concentration of 0.025 g/L was added. Absorbance was measured at 517 nm at the time interval of 0, 0.5, 1, 2, 5 and 10 min. The remaining amounts of DPPH radical were calculated from calibration curve. For comparison of radical scavenging activity of extracts, absorbance was measured at fifth minute.

**Metal chelating activity:** Fe²⁺ chelating activity was analyzed by 2, 2-bipyridyl competition assay method as described by Wu et al. [20]. The reaction mixture contained 0.25 ml of 1 mmol/L FeSO₄, 1ml Tris-HCl buffer (pH 7.4), 0.25 ml BGS extract, 0.4 ml of 10% hydroxyamine-HCl, 1mL 2,2-bipyridyl solution (0.1% in 0.2 mol/L HCl), and 2.5 ml ethanol. The final volume was made up to 6.0 ml with ethanol. The absorbance of mixture was measured at 522 nm and used to evaluate Fe²⁺ chelating activity using disodium ethylene diamine tetracacetate (Na₂EDTA) as a standard.

**Determination of total flavonoid content (TFC):** The TFC of BGS extracts of both varieties were determined according to the method as described by Zou and Wei [21]. 0.5 ml of sample solution dissolved in 2 mL of distilled water and afterwards with 0.15 mL of 5% NaNO₂ solution, 0.15 mL of 10% AlCl₃ solution was added after 6 min of incubation then allowed to stand for 6 min, followed by adding 2 mL of 4% NaOH solution to the mixture and made volume up to 5 mL, the mixture was mixed thoroughly and left the solution for 15 min. The absorbance was measured 510 nm. TFC was expressed in milligrams of equivalents per gram of BGS extract.

**Statistical analysis:** Three samples of each BGS variety were analyzed individually in triplicate and the results were reported as mean (n=3×3×1) ± SD (n=3×3×1). Statistical significance of the differences between mean values was assessed by 2 way analysis of variance (ANOVA) using Minitab 2000 Version 13.2 statistical software (Minitab Inc. Pennsylvania, USA). A probability value of p ≤0.05 was considered to denote a statistically significant difference [22].

**Results and Discussion**

**Proximate analysis**

Results of the proximate composition of both BGS varieties are shown in table 1. Significant (P<0.05) differences were observed between BGS-1 and BGS-2 varieties in their contents of moisture (22.91 and 29.32%, respectively). In contrast to our results, Ali et al. [8], and Nyam et al. [23], reported significantly lower moisture content (7.62-8.20% and 7.3% respectively) whereas Prashantha et al. [24], and Islam et al. [25], recorded 53-79.5% of moisture which may be attributed to variations in the genetic makeup or due to the collection of sample at different level of fruits maturity.

The oil contents (given as percentage to the dry weight) of both BGS were 31.51 and 40.79% respectively which is appreciably higher than 19.30 and 26.00% average oil as reported by Ali et al. [8], and Nyam et al. [23], respectively. However, the oil content in the BGS-1 was close to the oil content (41-45%) of the four different cultivars of BGS [26]. Our findings are also in good agreement with the findings of Airan and Shah [27], and Prashantha et al. [24], who reported the average yield of 35% and 40.45% of the BGS oil.

The total protein content was found to be 14.92 and 19.17% of which 5.01 and 3.91% were water soluble in BGS-2 and BGS-1 varieties respectively. The present results revealed that BGS is a potential rich resource of protein and could be used in various food applications. The protein content estimated by micro-Kjeldahl method showed a considerably higher value than that given by Lowry method. The reason is that Lowry method was applied to a water extract and took into accounts, in this case, the water soluble proteins only. Further, micro-Kjeldahl method takes into account both the protein and non-protein nitrogen. The amount of protein in BGS-1 was in good agreement
Physicochemical analysis of oil

Different physical and chemical parameters of both BGS oils are presented in table 2. A slight variation was observed in the density determined in BGS oils (0.9448 and 0.9605 mg mL⁻¹ at 25°C) as presented in table 2. A slight variation was observed in the density of BGS-2 and BGS-1 oils were 1.4698 and 1.4799 respectively, close to the BGS-2 oil (Table 3). Since a rich source of tocopherols BGS oil (99.74-110.07 mg/g) in BGS oil. A significantly (P < 0.05) higher percentage of unsaponifiable matters ranging from 1.07 to 1.79% which is in good agreement with the values 1.12-1.71% mentioned by Ali et al. [8]. The significantly (P < 0.05) lower percentage of unsaponifiable matters as obtained in the BGS-1 oil (1.07%) points to lower amounts of hydrocarbons, higher alcohols and sterols than those in the BGS-2 oil.

Analysis for oxidative stability of oils

Different parameters studied for the oxidative stability of the oils extracted from both BGS are given in table 2. The BGS oils displayed the peroxide values of 3.02-5.97 mEq/kg, lower than that of 6.13–8.50 mEq/kg reported by Ali et al. [8]. Both type of BGS oils contained p-Anisidine value in the range of 3.59-3.89, not significantly (P>0.05) different from each other. The specific extinctions at 232 and 270 nm were 4.32 and 0.52; 5.07 and 1.03 observed in BGS-2 and BGS-1 oils respectively.

Tocopherols analysis

Tocopherols are important biological antioxidants and have been correlated with the reduction of heart diseases, delay of Alzheimer’s disease, and preclusion of cancer. Especially, α-tocopherol (vitamin E) prevents oxidation of body lipids including polysaturated fatty acids and lipid components of cells and organelle membranes. The concentration of vitamin E (239.81-381.06 mg/g) was found to be highest followed by γ-tocopherol (52.85-61.09 mg/g) and δ-tocopherol (99.74-110.07 mg/g) in BGS oil. A significantly (P<0.05) higher concentration of vitamin E was observed in the BGS-1 oil as compared to the BGS-2 oil (Table 3). Since a rich source of tocopherols BGS oil can be used in food, feed, pharmaceuticals, cosmetics and resins.

Fatty acid (FA) analysis

FA compositions of BGS oils were determined by GLC and are presented in table 3. Except myristic and oleic acids, significantly differences (P<0.05) were observed in the remaining FAs in both BGS oils. BGS-2 and BGS-1 oils contain highest amount of oleic acid.
(48.14 and 44.05%) followed by stearic acid (25.44 and 29.14%), oleic (16.10 and 15.87%) and linoleic (6.87 and 5.41%) acids respectively.

Similar trend in the FA composition findings were also reported in the Chinese BGS oil [26]. However, a higher concentration of eleostearic acid was observed in the Chinese BGS oil as compared to our results. Contrary to our results, Nyam et al. [23], recorded mainly stearic (32.4%), oleic (1.5%) and linoleic acid (2.6%) in the same fruit. The fatty acid profiles (except stearic and eleostearic acid) evaluated in this study are in close agreement with the values reported by Ali et al. [8]. This variation in the fatty acid composition may be due to the variety, soil, and climatic conditions.

Antimicrobial activity

The antimicrobial activity of BGS oil of both varieties were evaluated against a set of microbes like Gram positive and Gram negative bacteria and selected fungal strains (Table 4). The oil obtained from both BGS exhibited different degree of antimicrobial activity against the all microorganism tested. Results obtained from disc diffusion method, followed by measurement of minimum inhibitory concentration (MIC), showed the following sensitivity order:

- Bacillus subtilis > Staphylococcus aureus > Pasturella multocida > Escherichia coli

The results indicated that Bacillus subtilis and Staphylococcus aureus were the most sensitive bacteria among selected bacterial strain tested by both BGS. In a previous study, BGS oil showed antibacterial activity against methicillin-resistant Staphylococcus aureus [29]. But this may be the first report regarding the antimicrobial activity of both BGS oil. A slightly higher antimicrobial activity was recorded for the oil extracted from BGS-2 than BGS-1. Similar pattern was also observed for MIC values (Table 4). A comparatively higher activity of both BGS oil was exhibited against Gram positive bacteria than Gram negative strains. The antibacterial activity of both varieties BGS oil was comparable with the standard drug, Amoxicillin.

The oil of both BGS varieties exhibited antifungal activity but less than standard drug (Flumequinene). The following sensitivity order of selected fungal strains was observed:

- Microsporum canis > Fusarium solani > Candida albicans > Aspergillus flavus

Our findings were similar to the results depicted by Braca et al. [30]. It can be inferred that the BGS oil has stronger and broader spectrum of antimicrobial activity. However, BGS oil is more potent against bacterial as compared to fungal strains.

Hemolytic activity

The BGS oil of both varieties was screened using a rapid assay against human and bovine erythrocytes. The results are summarized in table 5. No toxicity was observed by BGS-2 oil but very low hemolytic activity was recorded by BGS-1 oil (Table 5). Shu-fing and Ng [20] reported the hemolytic activity of steryl glycoside fraction from tubers of Momordica cochinchinensis. However, no previous report was available about the toxicity of Momordica Charantia.

Effects of solvent/technique on the extracts yields

Amounts (g/100g of dried BGS) of the antioxidant extract using three different solvents (water, ethanol and aqueous ethanol (ethanol: water, 80:20 v/v) and three extracting techniques: shaker, reflux and ultrasonic waves are shown in table 6.

The vigor of the extraction procedure mainly affected the amount of the antioxidant components extracted from BGS of both varieties which may probably vary from sample to sample. Amongst other contributing factors, efficiency of the extracting solvent to dissolve endogenous compounds might also be very important. For the effectiveness of extracting technique, the results showed that yields of the extract were better when extraction was done under ultrasound treatment followed by reflux and then shaking, regardless of the plant material and solvent used. This indicates that ultrasonic waves not only reduce the particle size of the extract.
Values (mean ± SD) are average of three samples of each BGS sample, analyzed individually in triplicate (n = 1x3 x 3), (P< 0.05) differences of means within the extracting solvent; Subscript letters within the same column indicate significant (P< 0.05) differences of means among the extracting technique.

Table 5: Hemolytic activity of bitter gourd seed (BGS) against human and bovine erythrocytes.

| Samples     | Extraction by shaker | Extraction by reflux | Extraction by ultrasound |
|-------------|----------------------|----------------------|--------------------------|
| BGS-1       | H₂O                  | EIOH                 | Aquous EIOH (80%)        |
| BGS-2       | 27.01 ± 1.9         | 22.37 ± 1.7          | 32.28 ± 2.0             |
| Extraction by reflex | 38.04 ± 3.4        | 28.32 ± 2.0          | 36.42 ± 2.0             |
| Extraction by ultrasound | 44.02 ± 1.9       | 32.71 ± 2.0          | 46.35 ± 2.0             |

Values (mean ± SD) are average of three samples of each BGS sample, analyzed individually in triplicate (n = 1x3 x 3), (P< 0.05) DW= dry weight. Superscript letters within the same row indicate significant (P<0.05) differences of means within the extracting solvent; Subscript letters within the same column indicate significant (P<0.05) differences of means among the extracting technique.

Table 6: Effect of solvent/technique on the total yield (g/100 g of DW) of BGS.

| Samples     | Extraction by shaker | Extraction by reflux | Extraction by ultrasound |
|-------------|----------------------|----------------------|--------------------------|
| BGS-H₂O    | 77.48 ± 3.83         | 66.02 ± 3.51         | 40.59 ± 1.69             |
| BGS-EIOH   | 37.52 ± 0.99         | 41.25 ± 2.11         | 70.31 ± 0.56             |
| Aquous EIOH (80%) | 82.01 ± 3.44 | 69.01 ± 3.18 | 68.11 ± 3.06 |
| Extraction by reflux | 38.19 ± 1.42 | 39.72 ± 1.38 | 70.62 ± 1.38 |
| Extraction by ultrasound | 41.13 ± 3.00 | 65.27 ± 2.99 | 78.11 ± 3.11 |
| BGS-H₂O    | 77.09 ± 3.41         | 65.17 ± 3.44         | 40.14 ± 2.03             |
| BGS-EIOH   | 38.18 ± 1.42         | 39.72 ± 2.48         | 70.62 ± 1.38             |
| Aquous EIOH (80%) | 81.14 ± 3.00 | 65.27 ± 2.99 | 78.11 ± 3.11 |
| Extraction by reflux | 41.13 ± 3.00 | 65.27 ± 2.99 | 78.11 ± 3.11 |
| Extraction by ultrasound | 41.13 ± 3.00 | 65.27 ± 2.99 | 78.11 ± 3.11 |

Values (mean ± SD) are average of three samples of each BGS sample, analyzed individually in triplicate (n = 1x3 x 3), (P<0.05); Superscript letters within the same row indicate significant (P<0.05) differences of means within the extracting solvent; Subscript letters within the same column indicate significant (P<0.05) differences of means among the extracting technique.

Table 6a: Total flavonoid and phenolic compounds of bitter gourd seed (BGS).
Samples | Extraction by shaker | IC50 values (mg/ml) | DPPH | Metal Chelation | BGS-H$_2$O | BGS-EtOH | Aquous EIOH (80%) | BGS-H$_2$O | BGS-EtOH | Aquous EIOH (80%) | BGS-H$_2$O | BGS-EtOH | Aquous EIOH (80%)
--- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---
BGS-H$_2$O | 144.10*±5.11 | 135.01*±5.01 | 371.62*±4.99 | 390.17*±4.91
BGS-EtOH | 157.42*±6.03 | 143.59*±3.72 | 442.18*±3.69 | 421.03*±3.99
Aquous EIOH (80%) | 163.52*±5.99 | 151.05*±5.11 | 461.27*±5.03 | 402.62*±4.15
--- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---
Extraction by reflux
BGS-H$_2$O | 143.02*±5.42 | 134.17*±4.82 | 372.19*±4.29 | 390.01*±5.12
BGS-EtOH | 157.01*±5.62 | 139.32*±4.17 | 444.16*±4.10 | 417.39*±3.62
Aquous EIOH (80%) | 160.28*±6.17 | 152.87*±4.92 | 455.17*±5.23 | 400.18*±5.12
--- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---
Extraction by ultrasound
BGS-H$_2$O | 50.01*±5.32 | 139.43*±5.28 | 370.16*±5.12 | 401.14*±5.20
BGS-EtOH | 166.14*±5.82 | 150.14*±4.21 | 443.18*±4.12 | 442.18*±3.71
Aquous EIOH (80%) | 168.28*±5.41 | 159.20*±4.99 | 465.13*±5.32 | 411.03*±4.21
--- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---
Values (mean ± SD) are average of three samples of each BGS sample, analyzed individually in triplicate (n = 1x3 x 3), (P < 0.05); Superscript letters within the same row indicate significant (P<0.05) differences of means within the extracting solvent; Subscript letters within the same column indicate significant (P<0.05) differences of means among the extracting technique.

Table 6b: DPPH and Metal Chelation of bitter gourd seed.

| Parameters (μg/g) | BGS-1 | BGS-2 |
| --- | --- | --- |
| Calcium | 399.81±6.99 | 425.08±12.03 |
| Copper | 2.89±0.06 | 3.51±0.07 |
| Iron | 49.21±1.02 | 43.06±1.04 |
| Zinc | 12.91±0.29 | 10.88±0.31 |
| Phosphorus | 135.92±3.86 | 146.19±3.04 |
--- | --- | --- |
Values (mean ± SD) are average of three samples of each BGS sample, analyzed individually in triplicate (n = 1x3 x 3), (P < 0.05).

Table 7: Mineral contents of bitter gourd seed (BGS).

highest DPPH scavenging activity. This reduction in the radical scavenging activity of the extracts, obtained by the reflux technique might be ascribed to the thermal decomposition of phenolics [35].

**Elemental analysis**

With regard to minerals, both BGS varieties contain relatively large quantities of calcium (425.08 and 399.81 μg/g) (Table 7) which is in close agreement with those (383.45–440.96 μg/g) reported by Ali et al. [8]. The amount of copper (3.51 and 2.89 μg/g) was higher when compared to the values described by Assubaie and El-Garawany [3]. Similar results for copper (2.85–3.52 μg/g) have been reported by Ali et al. [8]. In both BGS varieties, the iron (43.06 and 49.21 μg/g) contents were in good agreement with the findings (41.10–45.03 μg/g) of Ali et al. [8] and were appreciably higher than those for BGS (12.35–32.33 μg/g) [3]. The contents of zinc in both BGS varieties (10.88 and 12.91 μg/g) were slightly higher than those in BGS (7.76–10.50 μg/g) studied by Assubaie and El-Garawany [3]. An appreciably higher amount of calcium and phosphorus was observed in the BGS-2 as compared to BGS-1.

**Conclusion**

The findings imply that both BGS can be used as a potentially source of lipid, protein, unsaturated fatty acids (especially elaeostearic acid), minerals, tocopherols and antioxidants such as flavonoids and phenols. BGS-1 was found to have appreciably higher contents of lipid and protein as against the BGS-2. BGS oil of both varieties exhibited notable antimicrobial activity against selected bacteria and fungi strains, assessed by disc diffusion and measurement of minimum inhibitory concentration by micro dilution method. Aqueous ethanol exhibited better potential for antioxidant extraction under ultrasound effect. However, BGS-1 showed remarkably higher level of TPC and TFC as compared to BGS-2 under ultrasonic treatment. These antimicrobial and antioxidant activities of BGS may contribute to its therapeutic applications.

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