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REVIEW ARTICLE

Characterisation and antioxidant activity of sohphlang (*Flemingia vestita*), a tuberous crop

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Abstract This study aimed to assess the nutritional and antioxidant properties of sohphlang (*Flemingia vestita),* a tuber that is traditionally consumed in raw form in Meghalaya, a North-Eastern state of India. Cultivated sohphlang (CS) and market sohphlang (MS) flours were analysed for nutrient composition. The extracts of the flours from conventional-assisted-extraction (CAE), microwave-assisted-extraction (MAE) and ultrasound-assisted-extraction (UAE) were analysed for total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities (AA). CS flour exhibited good nutritional properties, TPC, TFC and AA than MS flour. Furthermore, MAE gave better extraction efficiency than UAE and CAE. HPLC results showed genistein as the predominant compound among the phenolic compounds identified that is comparable with soybean. Thus, tuber sohphlang that is eaten in raw form can serve as a versatile source of food and nutritional security to the people. The tuber also offers scope for exploiting its health benefitting functional ingredients.

Keywords Sohphlang (*Flemingia vestita*) · Ultrasound · Microwave · Conventional · Antioxidant · Isoflavones

Introduction

Depending on the geographical distribution and climatic conditions some plants which are found to be native or endemic to a particular area can have a great potential to be used as foods. The North-Eastern region of India holds very rich landraces in vegetation with diverse fruits, vegetables, rice, tuber crops etc. Many studies have been reported on the use of traditional fruits and vegetables of Meghalaya as medicinal plants (Laloo et al. 2006). However, adequate information through scientific studies about the nutritional aspects of most foods is still lacking.

Sohphlang (*Flemingia vestita)* belonging to the family Fabaceae is a wild indigenous edible tuber (Kayang 2007) or tuber like fruit. Commonly called as sohphlang (grassy fruit) by the Khasi tribe, it is eaten in raw form and commercially distributed among the Khasi and Jaintia Hills of Meghalaya, North-East India (Kayang 2007). It is a seasonal tuber crop with the availability period from mid-October to February. Sohphlang has been used by the natives of Meghalaya in local traditional medicine to cure worm infections (Das et al. 2009).

The tuber crops are used as a staple food in many developing countries. These tuber crops have great potential to provide an economical source of energy because of their considerable good source of nutrients (Chandrasekara and Kumar 2016). The tuber crops show antioxidant activities (Dilworth et al. 2012) and the presence of hydroxycinnamic phenolic acids such as chlorogenic acid, caffeic acid, coumaric acid and ferulic acid in potato and yam have been reported (Im et al. 2008; Wu et al. 2012; Zhang et al. 2018). The plant is also known to exhibit anti-inflammatory, anti-carcinogenic, anti-diabetic properties (Chandrasekara and Kumar 2016).

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The extraction of bioactive compounds from plant matrix by conventional methods like refluxing and mechanical shaking is both time and solvent consuming (Wu et al. 2012; Dahmoune et al. 2015). Thus, ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) have been introduced to improve the extraction efficiency (Wu et al. 2012; Dahmoune et al. 2015) with reduced consumption of solvent and shorter extraction time.

Other than traditional use for beneficial properties, sohphlang remains as an unexplored crop with respect to its nutrients and phytochemical content. This study, therefore, is aimed to evaluate the content of nutrients and phytochemicals and methods for extraction of phenolic compounds and estimate its antioxidant property. The results were further compared with those reported for potato, sweet potato, cassava, taro and yam (Dilworth et al. 2012; Wu et al. 2012; Aprianita et al. 2014; Omohimi et al. 2018).

Materials and methods

Chemicals and reagents

Analytical grade chemicals were purchased from Sisco Research Laboratory (Mumbai, India), Merck (Mumbai, India) and HiMedia (Mumbai, India). HPLC grade solvents and analytical grade ethanol were purchased from Merck (Mumbai, India) and Changshu Yangyuan Chemical (China) respectively. All standards were purchased from Sigma-Aldrich (St Louis, MO, USA) and TCI (Toshima, Kitaku, Tokyo, Japan).

Plant material

Cultivated sohphlang (CS) tuber was collected from the farm at Jongksha village of Meghalaya and market sohphlang (MS) tuber was procured from the local market of Meghalaya. The farmers soon after harvest bifurcate sohphlang, one part is taken to the markets and the other part is kept for storage. Sohphlang is stored for 1 month in dug pits in the field as seed stock and for future market purposes. As and when there is demand for sohphlang, the farmers dig the stored sohphlang for selling. In this manuscript, sohphlang tuber obtained directly after harvest has been termed as cultivated sohphlang (CS) while the one obtained from the market after 1 month of storage period has been termed as market sohphlang (MS).

Sohphlang tubers were sorted, trimmed to remove the defective parts, washed properly, cut into uniform cubes and dried in a tray dryer 40 °C for 10 h. The dried cubes were ground using a high speed laboratory mixer grinder (Philips HL Model No. 1632, India) for 2 min and finally passed through a 300 µm sieve. The powder was cooled and packed in air tight bags and stored at 4 °C until future analysis.

Chemical composition

Analysis of moisture, ash, fat, protein and carbohydrate was done following the protocols of AOAC Official Method (AOAC 2000). Total starch and total dietary fibre were determined using Starch (GO/P) Assay Kit and Total Dietary Fibre Assay Kit, respectively (Sigma-Aldrich, St Louis, MO, USA). Sohphlang flours were estimated for amylose content (Van Hung and Morita 2005); titrable acidity, reducing sugars and ascorbic acid were analyzed following Ranganna (2012). Cyanide content was analysed at Central Tuber Crop Research Institute, Kerala, India as per the method of Nambisan and Shanavas (2013).

Mineral content

Mineral components viz, potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), phosphorus (P), iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) were determined by atomic absorption spectrometry (Thermoscientific Model No. ICE3500, USA) as given in AOAC official method (AOAC 2000). The absorption spectrum of each mineral was determined at specific wavelength and calibration curve of external standards was used to calculate the concentration of each mineral. Phosphorus was analysed by molybdophosphoric blue colour test following Singh et al. (2005) and the estimates are expressed in mg/100 g.

Phenolic compounds extraction

Sohphlang tuber flours were extracted in 80% methanol with a sample: solvent ratio of 1:10 (w/v) using three different extraction methods. The first one was microwave assisted extraction (MAE), in which the sample-methanol mixture was heated at 450 W for 2 min in a Samsung Model No. C103FL, Thailand domestic microwave oven. The second one was ultrasound assisted extraction (UAE) in which the sample mixture was exposed to acoustic waves for 15 min at 30 ± 2 °C in pulsed mode (2 s ON followed by 2 s OFF) using 6 mm probe ultrasonicator (Takashi Ultrasonic Homogenizer Model No. U500, Japan). The third one was a conventional method of extraction (CAE) which involved shaking of the sample mixture in an incubator (Sartorius Cat No. 8864845, Germany) set at 150 rpm for 2 h. All extracts were cooled to room temperature, centrifuged at 2655×*g* for 10 min. The supernatant was then collected and passed through 0.45 µm filter (WHATMAN). The filtered extract was stored at 4 °C until further use.

Isoflavones extraction

For further confirmation of the presence of isoflavones in sohphlang, the flour samples were extracted in dimethyl sulphoxide:ethanol:water (5:70:25, v/v/v) (Luthria et al. 2007) following the same extraction methods and procedure followed for phenolic compounds.

Total phenolic content (TPC)

Total phenolic content in sohphlang tubers was estimated using Folin–Ciocalteu assay (Slinkard and Singleton 1977). Briefly, 10 mg of standard gallic acid was dissolved in 10 mL methanol and was then diluted to give appropriate concentrations (10–100 ppm) for a standard curve. For the analysis, 20 µL each of blank consisting of distilled water, gallic acid standard and tuber extract were taken in separate test tubes. To this was added 1.58 mL of distilled water followed by 100 µL of Folin–Ciocalteu reagent. The content was mixed well and after 8 min, 300 µL of sodium carbonate was added and vortexed immediately. The samples were incubated in the dark for 30 min at 40 °C before taking the absorbance at 765 nm in a UV–Vis spectrophotometer (CE 7400 Cecil, England). The results were expressed in mg gallic acid equivalent (GAE) per 100 g of sohphlang tuber on dry weight (DW) basis.

Total flavonoid content (TFC)

The flavonoid content was determined by aluminium trichloride method (Chang et al. 2002). Briefly, 0.5 mL of the extract, standard or blank was mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum trichloride, followed by 0.1 mL of 1 M potassium acetate and 2.8 mL of deionised water. After incubation at room temperature for 40 min, the absorbance of reaction mixture was measured at 415 nm against distilled water taken as blank in a UV–Vis spectrophotometer (CE 7400 Cecil, England). The concentration of TFC in the flour samples was calculated from calibration standard curve (10–100 mg/L) of methanolic quercetin standard and expressed as mg of quercetin equivalents (QE) per 100 g of tuber on dry weight (DW) basis.

Antioxidant activities

Scavenging activity against DPPH radical

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed following the methods of Brand-Williams et al. (1995). Briefly, 1.4 mL of 1×10^{-4} M DPPH in methanol was mixed with 100 µL of each test sample. For the blank, 1.4 mL of DPPH radical solution was mixed with 100 µL of methanol. The mixture was incubated for 30 min at 37 °C and the decrease in absorbance was measured at 515 nm. The antioxidant capacity of the extract was expressed as a percentage of inhibition of DPPH radical (% inhibition of DPPH radical) and was determined as follows:

$$\text{Percentage inhibition}\,[\%] = [(\text{Ao} - \text{As})/\text{Ao}] \times 100$$

where Ao is the absorbance value of the blank; As, is the absorbance of the sample extract. The effective concentration of sample required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis from the curve by plotting percentage inhibition versus concentration.

Scavenging activity against ABTS radical

ABTS assay works on the principle of the ability of antioxidants to interact with the $ABTS^{\cdot+}$ (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical cation. The scavenging activity against ABTS radical was determined following the method of Shalaby and Shanab (2013). Briefly, 7 mM ABTS and 2.45 mM potassium persulfate were prepared in ethanol and a radical solution was prepared freshly by mixing the above two solutions in the ratio of 1:1 (v/v) and left to stand in the dark at room temperature (27 °C) for 12–16 h. This solution was then diluted with ethanol to get an absorbance of 0.70 ± 0.05 at 734 nm. For the analysis, 0.9 mL of the diluted $ABTS^{\cdot+}$ radical solution was mixed with 0.1 mL of the extracted supernatant (for tested sample) and ethanol (for blank), and the absorbance was read at 734 nm in a UV–Vis spectrophotometer (CE 7400 Cecil, England) Cecil, England) against ethanol as blank after 15 min. The scavenging activity was calculated as the percent inhibition of absorbance at 734 nm using the equation below.

$$\text{Percentage inhibition}\,[\%] = [(\text{Ao} - \text{As})/\text{Ao}] \times 100$$

where Ao and As has the same connotation as before. The IC_{50} of the ABTS radical scavenging was also determined as described in DPPH radical scavenging activity.

Determination of ferric reducing antioxidant potential (FRAP)

The ferric reducing antioxidant power of sohphlang tuber flours was determined following the method of Benzie and Strain (1996). Briefly, reaction mixture was prepared by mixing 2.5 mL of a 10 mM 2,4,6-TPTZ [2,4,6-tri(2-pyridyl)-1,3,5-triazine] solution in 40 mM hydrochloric acid with 2.5 mL of 20 mM ferric chloride and 25 mL of 0.3 M acetate buffer (pH 3.6) and was then pre-warmed at 37 °C. To 300 mL of this reaction mixture, 40 µL of the standard samples/test samples were added. The reaction mixture was

incubated at 37 °C for 4 min and the absorbance was determined at 593 nm in a UV–Vis spectrophotometer (CE 7400 Cecil, England) against a blank that was prepared using distilled water. Different concentrations of ferrous sulphate (10–100 mg/L) were prepared so to get a standard curve and the ferric reducing power was expressed from ferrous sulphate standard curve as mg ferrous Fe(II) equivalents per 100 g of tuber on dry weight (DW) basis.

Determination of metal chelating capacity (MCC)

Metal chelating capacity was determined based on the method described by Santos et al. (2017). For the analysis, 0.2 mL sample was mixed with 1.0 mL of 0.125 mM ferrous sulphate and 1.0 mL of 0.312 mM ferrozine. Equilibration was allowed for 10 min at room temperature and the absorbance was recorded at 562 nm in a UV–Vis spectrophotometer (CE 7400 Cecil, England). The control contained all the reaction reagents except the extract. Metal chelating capacity was calculated as follows:

$$\text{Chelation activity } [\%] = [(\text{Ao} - \text{As})/\text{Ao}] \times 100$$

where Ao is absorbance of control blank, and As is absorbance of sample extract.

HPLC analysis of phenolic compounds

Standards preparation

The standards used for identification and quantification were ascorbic acid, gallic acid, catechin, chlorogenic acid, caffeic acid, ferulic acid, coumaric acid, rutinhydrate, quercetin, sinapic acid; and isoflavones: genistein, daidzein, biochanin-A, formononetin, daidzin and genistin. For preparation of the standards, 10 mg of the standards (except isoflavones) were dissolved in 10 mL methanol while 10 mg isoflavones were dissolved in 10 mL dimethylsulfoxide (DMSO). Calibration standard curves were obtained by diluting the stock solution with methanol and DMSO respectively to give the appropriate concentration range.

Identification and quantification of phenolic compounds

For identification and quantification of phenolic compounds present in sohphlang flours RP-HPLC (Waters system) gradient elution method of Saikia et al. (2015) was used. Binary pump (Waters, 1525), symmetry 300^{TM} C_{18} column (5 µm, 4.6 × 250 mm), and UV–Vis detector (Waters, 2489) were used for the separation of compounds. Elution of compounds was employed using mobile phase A consisting of acidified ultrapure water (0.1% acetic acid, pH 3.2,) and mobile phase B consisting of HPLC grade methanol as follows: 0–8 min (80% A), 9–12 min (65% A), 13–16 min (45% A), 17–20 min (30% A), 21–30 min (20% A), 31–34 min (10% of A) and washing of the column at 35–39 min (65% A) and finally at 40–45 min (80% A). Operating conditions were 0.8 mL/min flow rate, 20 µL injection volume and UV–Vis spectra were recorded at 254 nm wavelength. Identification and quantification of the compounds present were based on the comparison of their retention times and spectral data with those of the standards.

Identification and quantification of isoflavones

Isoflavones were analyzed by RP-HPLC following the gradient method described in Krenn and Potsch (2006) with modifications. Symmetry 300^{TM} C_{18} (5 µm, 4.6 × 250 mm) column with a binary pump (Waters, 1525) and a UV–Vis detector (Waters, 2489) were used. The mobile phases used however, were Millipore water (A) and acetonitrile (B) with 1 mL/min flow rate, 20 µL injection volume and at the wavelength of 254 nm. The gradient method followed was 8–25% B (0–15 min), 25–35% B (15–18 min), 35–45% B (18–23 min), 45–100% B (23–29 min), and then back to 8% B (29–40 min). For identification and quantification, the retention times and spectral data of the compounds present was compared with those of the authentic isoflavone standards.

Statistical analysis

The experiments were carried out in triplicates and the results are presented as mean ± standard deviation of mean. The data were statistically analysed using SPSS version 20. Analysis between samples were analysed by Paired-comparison t test ($p \leq 0.05$) while Duncan's multiple range tests at $p \leq 0.05$ significance level were carried out for analysis between extraction treatments. Correlation coefficient between antioxidant compounds (TPC, TFC) and antioxidant activities (DPPH, FRAP, MCC, ABTS) was determined using Pearson's correlation coefficient (SPSS version 20).

Results and discussion

Chemical composition

The nutrient composition of sohphlang (*Flemingia vestita*) tubers is presented in Table 1. The storage period, washing and skin peeling, transportation and exposure to sun during outdoor sale in the market might have caused a significant decrease ($p < 0.05$) in the moisture content of MS flour (10.58%) as compared to CS flour (11.22%). The moisture content of both CS and MS flours was compared with starch rich non cereal crops and was found to be within the

Table 1 Chemical composition of sohphlang flour samples

Parameters (%)	CS flour	MS flour
Moisture	11.22 ± 0.25[a]	10.58 ± 0.23[b]
Ash	2.12 ± 0.15[a]	1.51 ± 0.13[b]
Protein	7.86 ± 0.44[a]	7.35 ± 0.32[a]
Fat	0.71 ± 0.26[a]	0.78 ± 0.13[a]
Carbohydrate	78.09 ± 0.39[a]	79.78 ± 0.16[b]
Dietary fibre	7.07 ± 0.36[a]	7.39 ± 0.22[a]
Starch	67.95 ± 0.15[a]	64.61 ± 0.31[b]
Amylose	18.90 ± 0.17[a]	16.75 ± 0.16[b]
Reducing sugar	3.47 ± 0.83[a]	7.89 ± 0.65[b]
Acidity	0.15 ± 0.08[a]	0.19 ± 0.11[a]
Ascorbic acid	17.58 ± 1.12[a]	14.33 ± 0.92[b]
Cyanide	ND	ND

Values are expressed as mean ± standard deviation of triplicate assays. Means with different superscripts [a,b] within a row are significantly different ($p < 0.05$). All values were reported in % dry basis (db) except moisture which was reported as % wet basis (wb)

ND not detected

range of the moisture content reported in yam flour (Omohimi et al. 2018). Moisture content provides an index of storage stability. Thus, the relative low moisture content of both CS flour and MS flour can resist the microbial attack and can assure good shelf-life stability, if stored under low relative humidity.

No significant difference ($p > 0.05$) was observed in the protein, fat and dietary fibre content between CS flour and MS flour. The protein content of MS flour (7.35%) was lower than that of CS flour (7.86%). Values lower than those obtained in the present study were reported for taro, yam, sweet potato and cassava flours (Aprianita et al. 2014). Both the tested samples showed comparable low amount of fat (< 1%) and the data were in agreement with other studies that have been reported in yam flour (Omohimi et al. 2018). The low fat content of sohphlang flour would indicate that the chance of spoilage by rancidity during storage would be less. Nutritionally, it has also been claimed that a product can be labelled as a 'source of fibre' if it contains more than 3 g fibre/100 g food (European Commission 2012). Since both CS and MS flours contain more than 3% dietary fibre, they can be labelled as a 'source of fibre' and hence may have the potential to be used as functional food. There was a significant difference ($p < 0.05$) in ash and carbohydrate content of CS and MS flours. The increase in carbohydrate content of MS flour might be due to the synthesis or accumulation of sugars during storage of tuber as observed in potato tuber (Malone et al. 2006). The reported carbohydrate content was comparable with the values (78.48–81.80%) reported by Omohimi et al. (2018) in yam flour. The higher proportion of carbohydrate than protein content directly reflects the tuberous nature of sohphlang.

Sugar and starch contents of yam were the factors reported to have influenced the taste, texture and preference of the yam varieties by the consumers (Otegbayo et al. 2012). The significant reduction ($p < 0.05$) in starch content and significant increase ($p < 0.05$) in reducing sugars content of MS flour (64.61% starch and 7.89% reducing sugars) as compared to CS flour (67.95% starch and 3.47% reducing sugars) can be attributed to the fact that MS flour was prepared from sohphlang that has been stored for 1 month and starch may have undergone amylolytic hydrolysis into sugars during storage (Otegbayo et al. 2012). The values of starch content reported for both CS flour and MS flour samples were in-line with the values reported in taro and sweet potato flours (Aprianita et al. 2014), however, it was lower than those reported in cassava flour (77%) and Indonesian yam flour (70%) (Aprianita et al. 2014). CS flour (18.90%) possessed significantly ($p < 0.05$) greater amylose content than MS flour (16.75%). No significant variation ($p > 0.05$) was observed in the acidity level of both CS flour (0.15%) and MS flour (0.19%). Ascorbic acid of CS flour (17.58%) was significantly higher ($p < 0.05$) than MS flour (14.33%) and this can be attributed to the oxidation during adverse handling and storage of MS tuber. Cyanide which is known for its toxicity was not detected in both the flours.

Mineral content

Table 2 presents significant difference ($p < 0.05$) in the mineral composition of the flours. The genotypic differences, chemical composition of the soil in which they are grown and amount of water available may be the factors that contributed to the variation in the minerals content in

Table 2 Mineral composition of sohphlang flour samples

Mineral	CS flour (mg/100 g)	MS flour (mg/100 g)
Macro-mineral		
Potassium	872.63 ± 0.82[a]	856.56 ± 0.65[b]
Sodium	31.45 ± 0.92[a]	26.84 ± 0.65[b]
Magnesium	171.65 ± 0.50[a]	152.23 ± 0.47[b]
Calcium	31.15 ± 0.22[a]	25.96 ± 0.24[b]
Phosphorus	23.33 ± 0.47[a]	19.98 ± 0.15[b]
Na/K	0.036	0.030
Ca/P	1.33	1.34
Micro-mineral		
Iron	6.72 ± 0.27[a]	4.56 ± 0.52[b]
Copper	9.38 ± 0.29[a]	6.96 ± 0.42[b]
Manganese	7.97 ± 0.24[a]	4.93 ± 0.35[b]
Zinc	3.61 ± 0.16[a]	2.70 ± 0.17[b]

Values are reported as mean ± standard deviation (n = 3). For each macro-mineral and micro-mineral, means with different superscripts [a,b] within a row are significantly different ($p < 0.05$). All values were reported in % dry basis (db)

tubers (Oluwatosin 1998). Among the macro-minerals, potassium which has been designated by Food and Drug Administration (FDA) as a nutrient of "public health significance" (Wong et al. 2015) was found to be the most abundant mineral in both the flours followed by magnesium, sodium, calcium and phosphorus. In both the flours, copper was the predominant micro-mineral (Cu > Mn > Fe > Zn). RDA of Cu is 0.9 mg/day (USDA 2015), thus, sohphlang tubers are able to meet this RDA. Copper is an essential micro-mineral that plays critical roles in numerous metabolic processes (Dilworth et al. 2012). However, precautions have to be taken to avoid excess intake of sohphlang flour as excess of copper intake can interfere with iron transport and/or metabolism thus causing anemia and can also cause certain kind of diseases (Ralph and McArdle 2001).

Sodium is essential for maintaining body's blood pressure and fluid balance, while potassium helps in maintaining acid–base balance and involves maintenance of osmotic pressure of the body fluids. Thus, sodium to potassium ratio (Na/K) is of great concern in controlling high blood pressure. Yang et al. (2011) reported that Na/K < 1.0 is protective for reducing the risk of cardiovascular disease (CVD). In the present study, the computed Na/K ratios of CS flour (0.036) and MS flour (0.030) appeared favorable towards hypertension control. However, the Ca/P ratio of both the flours was lower than the recommended ratio of 2:1 (Adatorwovor et al. 2015). It is evident that consumption of sohphlang alone will not be sufficient for bone formation and modified foods rich in calcium should be consumed for compensation.

Total phenolic content (TPC)

TPC of CS and MS flours (Table 3) subjected to different extraction treatments showed significant variations ($p < 0.05$). Both sample flours (CS and MS) and extraction methods (UAE, MAE and CAE) significantly influenced the phenolic contents. Among the selected samples, CS flour significantly contained the highest amount of TPC than MS flour. Phenolic content for the CS flour samples extracted by UAE, MAE and CAE in the study ranged from 917.65 to 1189.41 mg GAE/100 g dry sample while that of MS flour it ranged from 776.67 to 940.52 mg GAE/100 g dry sample. Considering the methods used, extraction following MAE method yielded the highest amount of TPC followed by UAE and CAE. The TPC was in the order of MAE_CS > UAE_CS > MAE_MS > CAE_CS > UAE_MS > CAE_MS. Further, TPC was highest in CS flour than MS flour for all the three extraction methods. Wu et al. (2012) and Dahmoune et al. (2015) have also reported that MAE method was best for increasing the yield of polyphenols from different plant materials.

Total flavonoid content (TFC)

Flavonoids are a group of polyphenols consisting of anthocyanins, flavones, isoflavones, flavanones, flavonols and flavanols that are predominantly found in plants. As seen from Table 3, amount of TFC in CS and MS flour samples was significantly ($p < 0.05$) affected by the flour type and extraction methods. The TFC of the sohphlang tuber flours varied from 654.42 to 769.22 and 537.04 to

Table 3 TPC, TFC, antioxidant activities and IC_{50} of sohphlang flours extracted by MAE, UAE and CAE

Parameters	UAE		MAE		CAE	
	CS flour	MS flour	CS flour	MS flour	CS flour	MS flour
TPC (mg GAE/100 g)	1037.06 ± 3.85^{Aa}	908.04 ± 4.41^{Bd}	1189.41 ± 5.68^{Cb}	940.52 ± 6.19^{De}	917.65 ± 5.33^{Ec}	776.67 ± 4.76^{Ff}
TFC (mg QE/100 g	697.59 ± 2.91^{Aa}	628.31 ± 2.46^{Bd}	769.22 ± 2.38^{Cb}	645.56 ± 5.38^{De}	654.42 ± 3.12^{Ec}	537.04 ± 1.96^{Ff}
FRAP (mg $FeSO_4$/100 g)	896.33 ± 3.51^{Aa}	809.30 ± 7.37^{Bd}	927.83 ± 2.86^{Cb}	873.48 ± 3.72^{De}	785.10 ± 4.84^{Ec}	635.36 ± 4.06^{Ff}
DPPH (%)	84.62 ± 1.10^{Aa}	80.13 ± 1.51^{Bde}	87.04 ± 2.40^{Ca}	82.41 ± 2.04^{De}	79.07 ± 1.33^{Eb}	77.79 ± 1.31^{Edf}
ABTS (%)	85.71 ± 2.89^{Aa}	80.08 ± 2.52^{Bd}	83.02 ± 1.74^{Ca}	79.13 ± 1.83^{Dd}	78.07 ± 1.65^{Eb}	76.12 ± 1.63^{Ed}
MCC (%)	79.51 ± 2.05^{Aa}	70.37 ± 1.45^{Bd}	84.59 ± 1.97^{Cb}	74.07 ± 0.88^{De}	71.93 ± 2.82^{Ec}	69.41 ± 1.79^{Ed}
DPPH IC_{50} (mg/mL db)	0.305 ± 0.12^{Aa}	0.316 ± 0.08^{Ad}	0.287 ± 0.09^{Ca}	0.302 ± 0.05^{Cd}	0.341 ± 0.05^{Ea}	0.359 ± 0.07^{Ed}
ABTS IC_{50} (mg/mL db)	13.75 ± 1.06^{Aa}	17.49 ± 0.78^{Bd}	11.49 ± 1.12^{Ca}	16.29 ± 0.84^{Dd}	27.62 ± 0.91^{Ec}	39.72 ± 1.24^{Ff}
DPPH IC_{50} (mg/mL db) of ascorbic acid	0.0255 ± 0.02					
ABTS IC_{50} (mg/mL db) of ascorbic acid	8.75 ± 1.47					

Values are expressed as mean ± standard deviation (n = 3). Means with different capital superscripts $^{A, B; C, D}$ and $^{E, F}$ within a row showed the significant difference ($p < 0.05$) between CS flour and MS flour of UAE, MAE and CAE respectively. Means with different small superscripts $^{a, b, c}$ and $^{d, e, f}$ within a row showed the significant difference ($p < 0.05$) between the treatments (UAE, MAE and CAE) of CS and MS flours respectively

db dry basis

645.56 mg QE/100 g on dry basis for CS flour and MS flour, respectively. As flavonoids come under total phenolic content, the results of TFC given in Table 3 showed that about 64.67–71.13% in CS flour and 68.83–69.14% in MS flour samples is composed of total flavonoids content, while the rest may be composed of phenolic acids and other phenolic compounds. Like TPC, the amount of flavonoids in CS tuber flour and MAE extracted flour samples was higher than in MS flour and UAE and CAE extracted flour samples; and were in the order of MAE_CS > UAE_CS > CAE_CS > MAE_MS > UAE_MS > CAE_MS. As flavonoids have been used to prevent diseases associated with oxidative stress such as diabetes (Chandrasekara and Kumar 2016), consumption of sohphlang tubers will be beneficial to consumers.

Antioxidant properties

Both the samples possessed antioxidant activities, which were established by DPPH, ABTS, FRAP and MCC assays (Table 3). Among the three extraction methods MAE showed the maximum anti-oxidant activities in FRAP, DPPH and MCC while UAE was found to be higher for ABTS radical scavenging activity. For all the extraction methods, CS flour sample showed significantly ($p < 0.05$) higher amount of antioxidant properties than MS flour sample except for DPPH, ABTS and MCC of CAE extracted sample where no significant differences were observed. The higher values for CS flour sample may be because the flour was prepared from freshly harvested tubers while MS flour was obtained from the stored tubers.

Table 3 shows the IC_{50} values for DPPH and ABTS scavenging activities and the significant difference ($p < 0.05$) between CS and MS flours could be observed for ABTS only. The IC_{50} value for DPPH of sohphlang flour samples was comparable with the values reported by Dilworth et al. (2012) for yam varieties, sweet potato and potato flours (0.041–3.127 mg/mL db). Ascorbic acid is a well known free radical scavenger as it shows low IC_{50} value and was used as a reference standard. The obtained IC_{50} values of the sample extracts were compared with the calculated IC_{50} value of the reference ascorbic acid standard whose DPPH IC_{50} value was 0.0255 mg/mL dry basis and ABTS IC_{50} value was 8.75 mg/mL dry basis. Among the sample and extraction treatments, CS and MAE flour samples showed the lowest IC_{50} values for both DPPH and ABTS. Lower IC_{50} value of CS flour and MAE extracted flour samples indicate that a small amount of sample is required to produce 50% inhibition hence highlighting the potency of these samples as free radical scavengers. Furthermore, MAE showed the lowest IC_{50} value because of its greater extraction efficiency of total phenols and flavonoids (Table 3) thus, possessing high potential of scavenging free radicals than UAE and CAE.

Based on TPC, TFC, reducing power, scavenging activity and metal chelating potential, MAE was considered as the best method of extraction as is reported earlier in other plants (Wu et al. 2012; Dahmoune et al. 2015). The differences in extraction efficiency of the extraction methods may be due to the differences in the mechanisms involved.

Correlation study

A positive, strong and significant correlation was observed between TPC, TFC and antioxidant activities (Supplementary, Table S1). The best correlation was observed between TPC and TFC (r = 0.911) probably because flavonoids belong to phenolic compounds. Similarly, both TPC and TFC were found to be significantly and positively correlated (r = 0.716–0.886) with antioxidant activities (FRAP, DPPH, ABTS and MCC). Correlation between scavenging activity (DPPH and ABTS) and FRAP was also observed to be significantly positive. The correlation between DPPH and FRAP may be explained by the similar electron-transfer mechanism (Huang et al. 2005). Further, the redox potential of Fe(III)-TPTZ (0.70 V) is comparable with that of ABTS (0.68 V), and similar compounds react in both the assays thus, causing the positive correlation between ABTS and FRAP (Huang et al. 2005). Thus, the findings of this study showed that the phenolic compounds contribute significantly to the ability of sohphlang tuber in reducing ferric to ferrous ion, scavenging DPPH and ABTS radicals through hydrogen donation and metal chelation and are consistent with the reports of significant correlations in sweet potato flour (Huang et al. 2006).

RP-HPLC analysis of phenolic compounds

RP-HPLC identification and quantification of individual phenolic compounds in sohphlang flour extracts (Fig. 1, Table 4) was done based on the combination of retention times and the calibration curves of external phenolic standards (Supplementary, Fig. S1). The amount of identified compounds in CS flour and MAE extracted samples were significantly higher ($p < 0.05$) as compared to MS flour and other samples. The results are in accordance with the values reported in Table 3.

In this study, coumaric acid was the only phenolic acid that could be identified in UAE, MAE and CAE extracted flours of CS and MS sohphlang flours. On the other hand, phenolic acids like chlorogenic acid, ferulic acid, gallic acid, and p-coumaric acid have been identified in potato flour extract (Im et al. 2008; Wu et al. 2012) and Chinese purple yam flour extract (Zhang et al. 2018). Chlorogenic acid constitutes 90% of the phenolic compounds in potato (Im et al. 2008). Among the phenolic compounds present,

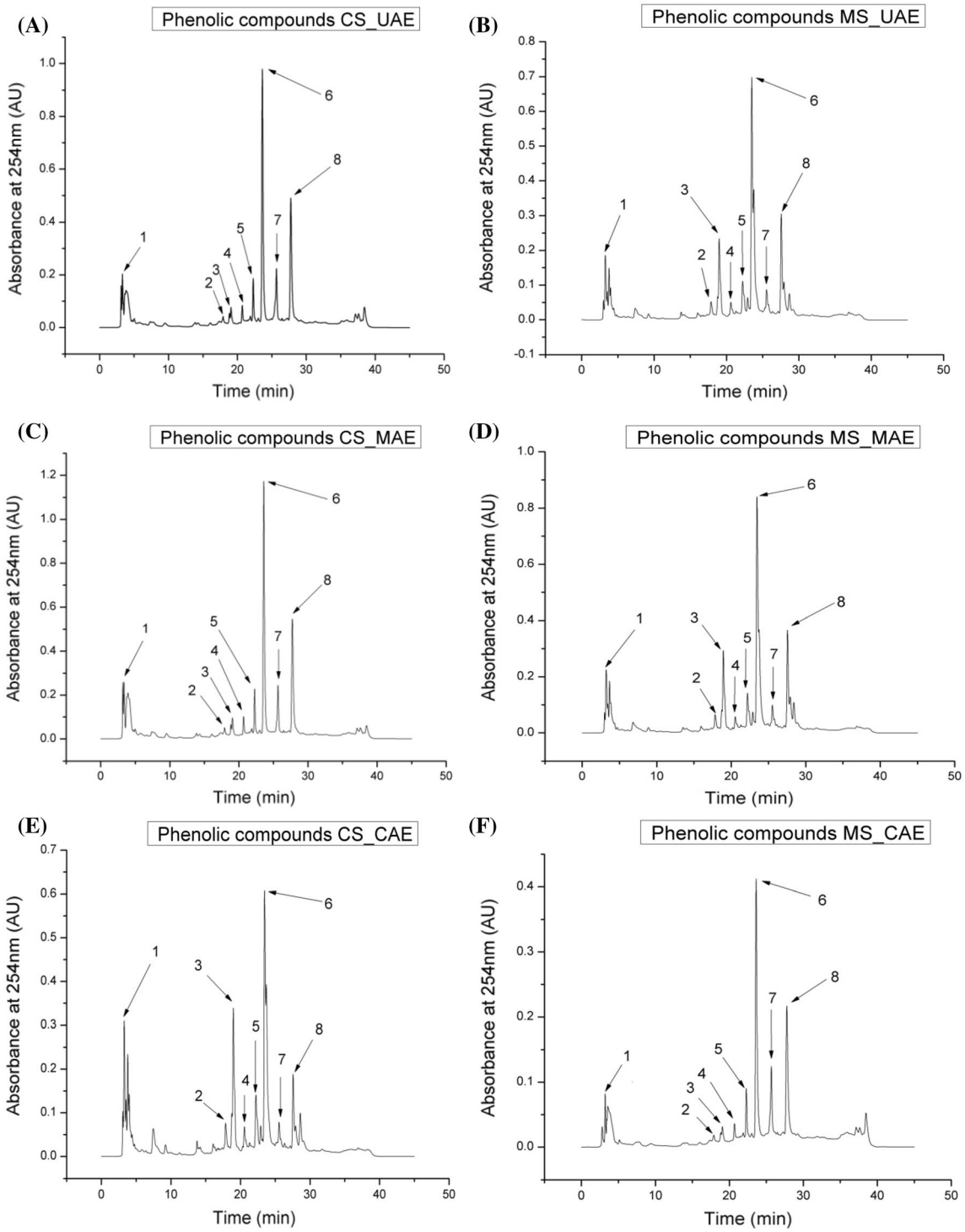

Fig. 1 HPLC chromatogram of phenolic compounds present in CS flour and MS flour extracts. **a** CS_UAE, **b** MS_UAE, **c** CS_MAE, **d** MS_MAE, **e** CS_CAE and **f** MS_CAE. Peaks: 1 = ascorbic acid, 2 = daidzin, 3 = coumaric acid, 4 = rutinhydrate, 5 = quercetin, 6 = genistein, 7 = formononetin and 8 = biochanin-A

genistein was found to be the predominant compound in both CS and MS samples and for all the extraction methods. As can be seen from HPLC chromatograms daidzin, rutinhydrate, quercetin, genistein, formononetin and biochanin-A were the flavonoids that were identified in all the samples irrespective of extraction methods. These findings were comparable with the TPC value reported in Table 3 which showed that about 64–71% TPC in sohphlang is composed of flavonoids.

Table 4 Identification and quantification of identified compounds in sohphlang flour extract samples

Peak no.	Compounds	Retention time (min)	UAE (μg/g)		MAE (μg/g)		CAE (μg/g)	
			CS flour	MS flour	CS flour	MS flour	CS flour	MS flour
1	Ascorbic acid*	4.41	52.17 ± 0.11^{Aa}	50.67 ± 0.23^{Bd}	63.73 ± 0.19^{Cb}	63.33 ± 0.08^{De}	83.66 ± 0.34^{Ec}	19.33 ± 0.16^{Ff}
	Phenolics							
2	Daidzin	17.96	12.20 ± 0.09^{Aa}	8.05 ± 0.22^{Bd}	13.81 ± 0.13^{Cb}	9.06 ± 0.41^{De}	14.22 ± 0.50^{Ec}	4.05 ± 0.26^{Ff}
3	Coumaric acid	19.05	5.66 ± 0.35^{Aa}	51.09 ± 0.07^{Bd}	24.36 ± 0.12^{Cb}	71.15 ± 0.18^{De}	86.70 ± 0.39^{Ec}	3.17 ± 0.27^{Ff}
4	Rutinhydrate	19.90	23.53 ± 0.13^{Aa}	7.61 ± 0.25^{Bd}	27.85 ± 0.16^{Cb}	5.13 ± 0.07^{De}	15.70 ± 0.17^{Ec}	4.01 ± 0.26^{Ff}
5	Quercetin	22.66	25.89 ± 0.04^{Aa}	21.04 ± 0.12^{Bd}	29.27 ± 0.14^{Cb}	23.72 ± 0.07^{De}	24.67 ± 0.23^{Ec}	22.93 ± 0.10^{Ff}
6	Genistein	23.77	63.36 ± 0.41^{Aa}	34.12 ± 0.16^{Bd}	96.47 ± 0.06^{Cb}	79.40 ± 0.17^{De}	77.80 ± 0.12^{Ec}	60.44 ± 0.07^{Ff}
7	Formononetin	25.79	50.60 ± 0.15^{Aa}	27.52 ± 0.08^{Bd}	56.21 ± 0.19^{Cb}	28.93 ± 0.07^{De}	22.77 ± 0.22^{Ec}	36.18 ± 0.26^{Ff}
8	Biochanin-A	27.82	34.94 ± 0.05^{Aa}	36.66 ± 0.10^{Bd}	76.16 ± 0.11^{Cb}	44.06 ± 0.08^{De}	16.54 ± 0.19^{Ec}	24.58 ± 0.13^{Ff}
	Isoflavones[#]							
1	Daidzin	12.62	160.11 ± 0.12^{Aa}	135.25 ± 0.45^{Bd}	485.40 ± 0.27^{Cb}	165.14 ± 0.07^{De}	325.07 ± 0.16^{Ec}	108.75 ± 0.11^{Ff}
2	Formononetin	25.53	99.11 ± 0.12^{Aa}	60.91 ± 0.33^{Bd}	313.37 ± 0.11^{Cb}	84.90 ± 0.06^{De}	86.64 ± 0.09^{Ec}	49.51 ± 0.29^{Ff}
3	Biochanin-A	26.48	258.14 ± 0.17^{Aa}	211.35 ± 0.10^{Bd}	602.38 ± 0.20^{Cb}	360.05 ± 0.09^{De}	200.92 ± 0.12^{Ec}	190.81 ± 0.15^{Ff}
4	Genistein	29.86	1983.40 ± 0.14^{Aa}	1261.11 ± 0.22^{Bd}	2113.40 ± 0.43^{Cb}	1569.58 ± 0.29^{De}	1643.47 ± 0.38^{Ec}	1252.92 ± 0.21^{Ff}

Values are reported as mean ± standard deviation (n = 3). Means with different capital superscripts [A, B; C, D] and [E, F] within a row showed the significant difference ($p < 0.05$) between CS flour and MS flour of UAE, MAE and CAE respectively. Means with different small superscripts [a, b, c] and [d, e, f] within a row showed the significant difference ($p < 0.05$) between the treatments of CS and MS flours respectively

*Vitamin C

[#]Dilution factor of 1.5 was used for isoflavones extracts of MS flour sample

RP-HPLC analysis of isoflavone compounds

RP-HPLC identification profiles of all the extracts were similar regardless of the sample types and extraction methods (Fig. 2), however, variations were observed in the amount of identified isoflavones present (Table 4). The retention times, spectral data and calibration curves of different isoflavone standards (Supplementary, Fig. S2)

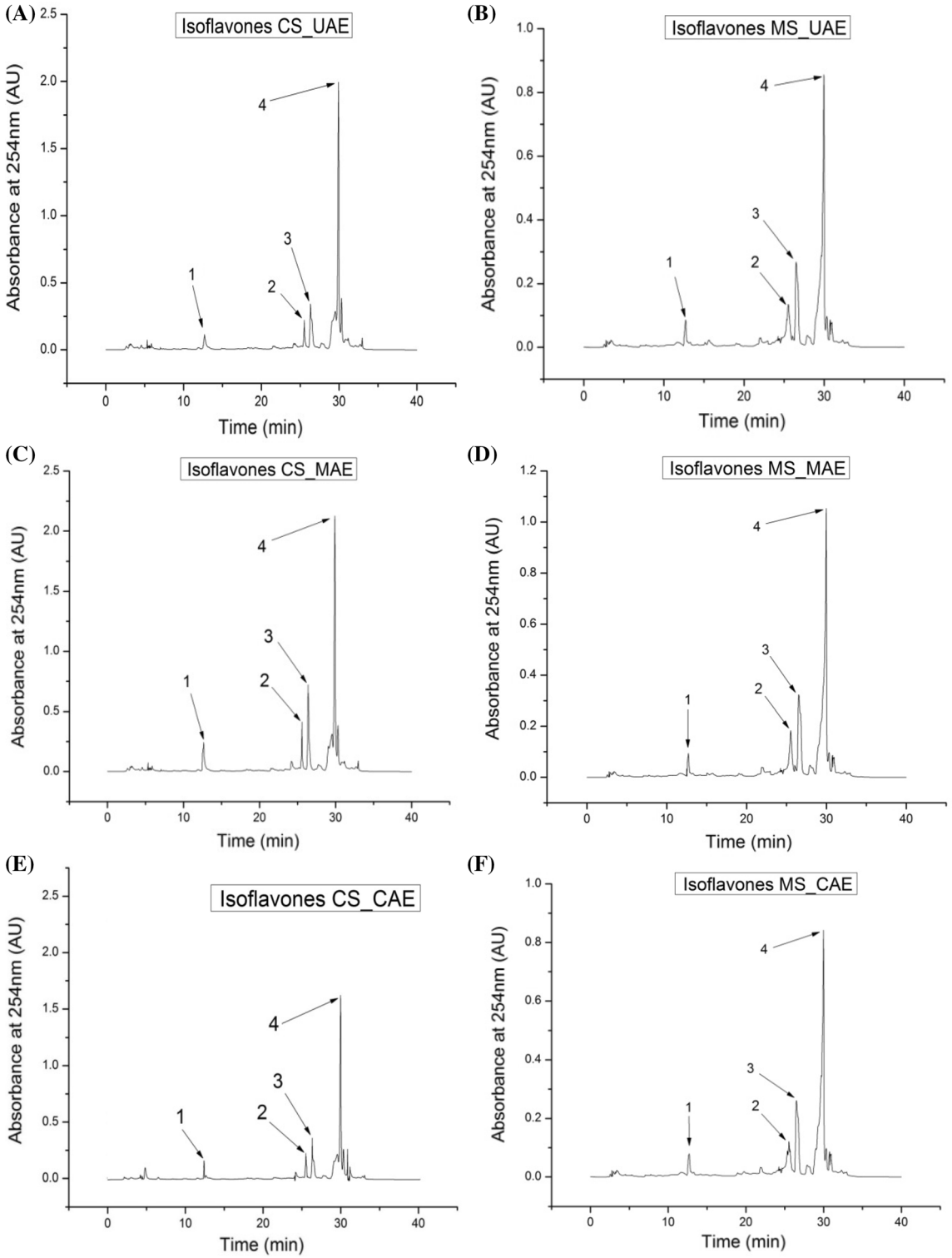

Fig. 2 HPLC chromatogram of isoflavones present in CS flour and MS flour extracts. **a** CS_UAE, **b** MS_UAE, **c** CS_MAE, **d** MS_MAE, **e** CS_CAE and **f** MS_CAE. Peaks: 1 = daidzin, 2 = formononetin, 3 = biochanin-A and 4 = genistein

were used for the identification and quantification of individual isoflavone compounds in sohphlang flour extracts. Isoflavones are the major phytochemicals found mainly in the plants belonging to family Fabaceae. For all the quantified isoflavones significant difference ($p < 0.05$) was observed in all the samples. Furthermore, CS flour and MAE extracted flour samples gave the maximum amount of isoflavones than MS flour and other extract samples. The major predominant isoflavones identified by HPLC in the flour samples was genistein followed by biochanin-A, daidzin and formononetin. It has also been reported that biochanin-A and formononetin usually gets converted to more potent genistein and daidzein after 4′-*O*-demethylation (Tolleson et al. 2002). This might cause an enhancement in the amount of genistein in sohphlang flours. The findings in the present study were in accordance with the findings of Rao and Reddy (1991) which also outlined the presence of formononetin and genistein in sohphlang although their quantification was not reported.

Isoflavones particularly genistein have been reported to confer health promoting properties by acting as anti-oxidant, anti-diabetes and anti-cancer even though they are not formally known as nutrients. Genistein is one of the primary isoflavones found in soybeans (Peñalvo et al. 2004). Genistein extracted from sohphlang has been demonstrated to be highly effective against intestinal parasites such as the poultry cestode—*Raillietina echinobothrida*, sheep liver fluke—*Fasciola hepatica* (Das et al. 2009), indicating its high potency as an anti-helmintic agent. The concentration of genistein reported in this study (1.252–2.113 mg/g) was comparable with the value (0.10–2.13 mg/g) in soy products as reported by Peñalvo et al. (2004). Thus, sohphlang can act as a vehicle to carry isoflavones mainly genistein, and consumption of sohphlang flours could, therefore, economically supply the required amount of genistein in the body especially for those who usually do not consume soy and soy products.

Conclusion

This study shows that cultivated sohphlang (CS) flour possessed considerable good amount of nutrients, and exhibited higher phenolic content and better antioxidant activities than market sohphlang (MS) flour sample. Among the three methods investigated for extraction of phenolic compounds from sohphlang flours, MAE was found to be the most efficient and less time consuming extraction method. HPLC analysis of the flour extracts showed the presence of an appreciably good amount of phytochemicals especially isoflavones that compares well with soybean. Cooking process is known to reduce the level of nutrients, phytochemicals and their biological activities. Since sohphlang is edible in raw form, the availability of nutrients and phytochemicals particularly the isoflavones from sohphlang is expected to be high and will provide physiological health benefits to consumers. Thus, sohphlang can act as a versatile crop for food and nutritional security to people and offers prospect of its utilization in new food products as a source of functional ingredients.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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