

Proximate composition, antioxidant activities and phenolic composition of *Cucumis sativus* forma *hardwickii* (Royle) WJ de Wilde & Duyfjes

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Abstract

In the present investigation, nutritional, anti-nutritional and antioxidant potential of *Cucumis sativus* forma *hardwickii* was evaluated for the first time. The results revealed that leaf and fruit of *Cucumis sativus* f. *hardwickii* were rich source of ash, carbohydrate, fat, fibre and proteins. In addition, appreciable amount of Vitamin C and E were also observed. Considerable antinutritional factors in leaf and fruit were noted which can be reduced by different processing methods. In sequential solvent extraction, methanol found to be the best in extraction of antioxidants which was supported with the highest antioxidant activities. Among all the extracts, it was noted that leaves extracted with methanol had higher amount of phenols and flavonoids than fruit. In addition, the potent radical scavengers mainly phenolics were detected using RP-HPLC where tannic acid, gallic acid and hydroxybenzoic acid were also quantified in leaf and fruit. Superior antioxidant potential may be due to presence of other bioactive compounds present in fruit. Additional research on bioactive properties of this plant is needed to explore this neglected wild cucumber for nutraceutical purpose and as a functional food.

Keywords: *Cucumis sativus* forma *hardwickii*, Proximate, Antioxidant, High-performance liquid chromatography

Introduction

Nutrition is the basic need to ensure better health and development. Nutrient deficiency and/or over sufficiency cause various disorders such as diabetes, obesity, cardiovascular diseases, cancer [1]. The various epidemiological studies have been proved that balanced diet along with fruit and vegetables may reduce risk of many chronic diseases [2]. In order to meet optimum nutritional requirement, there is urgent need to explore wild plant species as a novel source of food. Across the world various geo-communities uses series of wild plant species to meet their daily requirement of dietary energy. These wild resources are the best source of nutrient and bioactive compounds. In addition, it is also an important source of income to hunters, gatherers and rural people [3]. Exploring these wild plants as a source of functional foods assist us to alleviate malnutrition, hunger and illness within society. Such wild plants with high nutritional and low antinutritional potential are the prerequisite while deciding the functional foods. The analysis of proximate composition, ant nutritional factor and natural phytochemicals help us to understand their nutritional significance to explore this neglected wild plants as a novel nutraceuticals.

Reactive oxygen species (ROS) are formed in body as part of normal cell metabolism but excess of ROS causes oxidative stress

related diseases such as arthritis, diabetes, arteriosclerosis, cardiovascular, cancer, neurodegenerative disorders and aging[4]. Antioxidants are the molecules that provide protection to living organisms from oxidative damage and their subsequent consequences. The synthetic antioxidants are highly used to scavenge ROS but it has several side effects. Nowadays, interest has been focused mainly on searching plant based antioxidants due to their non toxic and safe nature. The wild plants are the significance source of antioxidant compounds which is effectively prevents oxidative damage [5, 6]. Hence, there is need to explore these wild, underutilized and neglected plants as a source of natural antioxidants.

In India, a number of major and minor cucurbits are cultivated which share about 5.6 % of the total vegetable production. Cucurbits are cultivated on about 4,290,000 ha with the productivity of 10.52 t/ha but we need to produce more vegetables to meet the minimum requirement of at least providing 300 g of vegetables/day/capita [7]. This target can only be achieved through combined use of growing improved varieties as well as utilization of the unexplored wild genotypes. *Cucumis sativus* forma *hardwickii* (family - Cucurbitaceae) is considered as wild progenitor of Cucumber. It is distributed from peninsular India to Sri Lanka as a wild plant [8]. It possesses easy cross ability with Cucumber and having multiple fruiting and branching habits. *Cucumis sativus*

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(Cucumber) is widely cultivated for their delicious fruits. The high amount of carbohydrate and some level of protein, fat and minerals have been recorded in fruits [9, 10]. *C. sativus* contain bioactive molecules such as phenols, flavonoids, terpenoids, tannin, saponin and glycosides which are responsible for various biological properties. The different parts such as seeds, leaves, fruits showed various pharmacological activities such as antioxidant, antiulcer, antimicrobial, anti-inflammatory [11,12,13], antidiabetic, hypolipidemic [14], analgesic, cytotoxic and antifungal [15,16]. While mining the data from the resources, work has been focused on *C. sativus* (Cucumber) but no information is available on its wild progenitor *Cucumis sativus* f. *hardwickii*. Therefore, attempt has been made to evaluate nutritional, antinutritional status, antioxidant properties and phenolic profiling of this wild and unexplored wild cucurbit.

Materials and Methods

Collection of plant materials

The leaves and fruits of plant were collected freshly during months of August 2015 from Panhala, Kolhapur, Maharashtra, India. The location lies between N16°48'32.38" Latitude and E74°6'33.11" Longitude. The plant was identified and a specimen was deposited at Herbarium of Department of Botany, Shivaji University, Kolhapur, Maharashtra, India (Voucher specimen No. UAA01). Collected materials were washed, separated into leaves and fruits and dried in hot air oven at 50 C for 72 h. The oven dried materials were powdered and further used for sequential extraction.

Preparation of plant extracts

The powdered samples of leaf and fruit were extracted sequentially with organic solvents such as hexane, chloroform, methanol using soxhlet apparatus. Finally material was macerated with constant stirring for 24 h and water extract was filtered. The solvent extracts were evaporated to dry at room temperature and dissolved in known volume of respective solvent and used for antioxidant assays.

Proximate composition

The moisture, ash, crude fat and crude fibre, vitamin C and E content and energy value of leaf and fruit were estimated by method of Association of Official Analytical Chemists [17]. The moisture content was determined by taking weight of sample before and after drying in hot air oven at 50 C for 24 h. Total ash content was estimated by incineration of 2.0 g of sample in muffle furnace at 600 C for 6 h and expressed in percentage. The amount of crude fat was determined by extracting known amount of sample with petroleum ether using Soxhlet apparatus. Acid and alkaline

digestion method was used to estimate crude fibre. The crude protein content was determined as described by Lowry et al. [18]. The carbohydrate content was calculated by subtracting the total of the percentages of crude protein, crude lipid, crude fibre, and ash on moisture-free basis from 100 [19]. Total carotenoids content was quantified by spectrophotometric method [20]. The energy value of fruit and leaf was calculated (K cal) by multiplying the amount of protein, carbohydrate and fat by the factors of 4, 4 and 9 (K cal). All the analyses were done in triplicate and results are expressed on dry weight (DW) basis.

Analysis of antinutritional factors

The antinutritional compounds such as phytic acid, tannins, nitrate and trypsin inhibitor content were determined by standard methods. The phytic acid was measured according to the methods of Gao et al. [21]. In brief, 0.5 g of fine powder was mixed with 10 mL 2.4 % HCl and stirred constantly at 220 rpm for 16 h. Centrifuge the mixture at 1000 g at 10 C for 20 min and crude extract was mixed with 1 mL of Wade reagent (0.03 % FeCl₃.6H₂O and 0.3 % sulfosalicylic acid in distilled water). Reaction mixture was centrifuged at 1000 g at 10 C for 10 min. Collected supernatant was used for measuring absorbance at 500 nm. A standard curve was obtained by using various concentration of sodium phytate (10-50 µg) as a standard and amount of phytic acid was expressed in percentage. The vanillin-HCl method was adopted to estimate tannin content [22]. The various concentration of catechin (50-300 µg/mL) was used to plot calibration curve and amount of tannin content was expressed in percentage. The nitrate content was determined by following method of Cataldo et al. [23]. The quantity of nitrate was calculated using standard curve prepared using different concentrations of KNO₃ (20-100 µg) and expressed in percentage. Trypsin inhibitor activity was evaluated using the method described by Kakade et al. [24] using benzoyl-DL-arginine-pnitroanilide (BAPNA) as substrate. Trypsin inhibitor activity was expressed as trypsin inhibitor unit (TIU)/mg protein content.

Phytochemical assays

Total phenolics content

Total phenolics content was estimated by using Folin-Ciocalteu method described by Singleton and Rossi [25]. A known volume sample extract was mixed with 1 mL of pre-diluted Folin and Ciocalteu reagent (1:10). After 5 min incubation at room temperature, 0.8 mL of (7.5% w/v) of sodium carbonate was added. The reaction mixture was kept at room temperature for 60 min. The absorbance was measured at 765 nm using UV-visible spectrophotometer (Jasco V-730, Japan). Tannic acid solution (20-120 µg/mL) was used to plot calibration curve. The amount of total



phenolics was expressed as mg tannic acid equivalent (TAE)/ g extract.

Total Flavonoids Content

Total flavonoids content was determined according the method of Sakanaka et al. [26]. Plant extract (50 μ L) was taken and diluted (0.5 mL) with distilled water followed by addition of 75 μ L of 5 % NaNO₂ solution. After 6 min incubation, 150 μ L of 10 % aluminium trichloride solution was added and kept mixture at room temperature for 5 min. Further, 0.5 mL of 1 M sodium hydroxide was added and final volume made up to 2 mL with distilled water. The absorbance was measured immediately at 510 nm. The various concentration of catechin (50-300 μ g/mL) was used to plot standard curve. The amount of total flavonoids was expressed as mg catechin equivalent (CE)/ g extract.

In Vitro Antioxidant Assays

DPPH Radical Scavenging Activity

DPPH radical scavenging activity was determined by following method of Brand-Williams et al. [27]. Methanolic solution of DPPH (0.025 g/L) was prepared freshly. A known volume of plant extract of different concentration (50-400 μ g/mL) was added to 1.0 mL of DPPH solution. Reaction mixtures were kept in dark for 30 min and decrease in absorbance was measured at 515 nm. IC₅₀ values of the extract i.e. concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

ABTS + radical scavenging activity

ABTS + radical scavenging activity was carried out using method of Re et al. [28]. ABTS + radical cation produced by reacting 7 mM aqueous solution of 2, 2' Azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) disodium salt with 2.45 mM potassium persulfate (final concentration). Further reagent mixture was allowed to stand for 12-16 h at room temperature. The reagent solution was diluted in ethanol (1:89) and equilibrated at 30 C to give absorbance at 734 nm of 0.7 \pm 0.02. Plant extract of different concentration (50-400 μ g/mL) was combined with 3 mL of ABTS + diluted solution. Absorbance was measured at 30 C exactly after 30 min at 734 nm. Radical scavenging activity was measured using a standard curve obtained from various concentrations of trolox standard solution (20-120 μ g/mL) and results were expressed as mg trolox equivalent (TE)/g extract.

Metal Chelating Activity

The chelating of ferrous ion was estimated by method of Dinis et al. [29]. About 0.5 mL (400 μ g/mL) plant extract was mixed with 50 μ L of 2 mM FeCl₂. The reaction was initiated by adding 100 μ L of 5 mM ferrozine. The final volume of reaction was made to 1.5 mL by adding distilled water and mixture was shaken vigorously. After 20 min incubation the absorbance was recorded at 562 nm. The chelating activity was measured using a standard curve obtained from various concentrations of ethylenediamine tetraacetic acid (EDTA) and expressed in mg of EDTA equivalent (EE)/g extract.

Ferric reducing antioxidant property assay (FRAP)

The antioxidant potential of solvent extracts was examined according the protocol of Benzie and Strain [30]. FRAP reagent prepared freshly and incubated at 37 C prior to use. FRAP reagent contained 2.5 mL of 10 mM 2,4,6- tripyridyl-2-triazine solution (TPTZ) in 40 mM HCl, 2.5 mL of FeCl₃.6H₂O and 25 mL of 300 mM acetate buffer (pH 3.6). A known volume of plant extract 0.5 mL (400 μ g/mL) was allowed to react with 2 mL FRAP reagent and incubated at 37 C for 30 min. The increase in absorbance was measured at 593 nm. Different concentration of FeSO₄.7H₂O (20-120 μ g/mL) was used to prepared calibration curve. Results were expressed in mg Fe (II) /g extract.

Phosphomolybdenum Assay

Total antioxidant activities of all solvent extracts were estimated by phosphomolybdenum method described by Prieto et al. [31]. Aliquot of plant extract of different concentration (400 μ g/mL) was mixed with 1 mL reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in water bath at 95 C for 90 min. After cooling the samples, absorbance was taken at 695 nm against blank. A calibration curve was plotted using standard solution of ascorbic acid (20-120 μ g/mL). Total antioxidant activity was expressed as mg ascorbic acid equivalent (AAE)/ g extract.

Analysis of phenolic compounds by Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC)

Sample preparation

The dried powder of leaf and fruit (100 mg) was mixed separately with methanol (10 mL) then mixture was kept on orbital shaker incubator at 110 \pm 2 rpm (25°C, 48 h). Further mixture was filtered through Whatman No 1 and centrifuged at 10,000 rpm for 20 min at 4°C. Finally methanolic extract of sample was stored at 4°C until HPLC analysis.



Qualitative and quantitative analysis of phenolic compounds

Detection and quantification of selected phenolic compounds were performed on reversed phase-high performance liquid chromatography (RP-HPLC) system (Shimadzu Model no. LC-20AD) coupled with a diode array detector and a Waters, Nova-Pak C18 column (4 μ m, 4.6 250 mm). The mobile phase consisted of water: acetonitrile: glacial acetic acid (90:5:5) with 1 mL/min flow rate and 20 μ L injection volume. The peaks were monitored at 280 nm with 15 min as runtime. The qualitative analyses of phenolic compounds in extracts were performed by comparing their retention times with those of standards. A standard curve of gallic acid (GA), tannic acid (TA), hydroxybenzoic acid (HBA), vanillic acid (VA), coumeric acid (CA) (Sigma-Aldrich, St. Louis, MO, USA) with four different concentrations (0.02–0.2 mg) were prepared for quantification of identified phenolic compounds and its expressed as micrograms per gram of dry weight (mg/g DW). Before HPLC analysis, all solutions (mixed standards, sample, and spiked solutions) were filtered through 0.22 μ m nylon syringe filter.

Statistical analysis

All the analyses were performed in triplicate and results are expressed as mean \pm SE. The data were analyzed by one-way analysis of variance (ANOVA) using the statistical software statistical package for social science (SPSS, version 16.0). The one-way analysis of variance (ANOVA) followed by the Duncan multiple range test was employed and the differences between individual means and each solvent used were deemed to be significant at $P < 0.05$.

Results and Discussion

Proximate composition

The results of proximate composition of leaf and fruit are presented in Table 1. The fruits showed higher (92.75%) moisture content than the leaves. Franca *et al.* [32] reported 97.8% moisture content in fruits of *Cucumis sativus* which was higher than *C. sativus* f. *hardwickii*. Our results are in good agreement with the leaves and fruits of *Cucumis dipsaceus* [33, 34]. The ash content is representing the minerals content of sample and which are essential for normal growth and development of brain, muscle, teeth, bone. In our study, appreciable amount of ash was found in both leaf (20.50%) and fruit (11.90 %) which was higher than *Cucumis sativus* [32, 10] hence it may be the rich source of minerals. The macro nutrients such as protein, carbohydrate and fat are very essential for energy and normal growth and development. In present study high amount of crude protein were noted in leaf (5.71 %) as compared to fruit (1.77%). Waziri and

Saleh [10] recorded 2.06 % crude protein content from cucumber fruit. A significant amount of protein content (17.25%) was noted in cucumber fruit by Franca *et al.* [32]. Chandran *et al.* [33] and Nivedhini *et al.* [34] reported appreciable amount of crude protein in both leaf (10.8 %) and fruit (8.59 %) of *Cucumis dipsaceus*. In our investigation, we noted least amount crude protein in the fruit of *C. sativus* f. *hardwickii* as compared to previous reports. The noticeable difference was found for carbohydrate content of leaf and fruit of *C. sativus* f. *hardwickii*. Our results revealed that leaf carbohydrate was higher (53.40 %) than the fruit (32.27%). The observed value of carbohydrate content indicates that fruit is good source of energy. The results are in good accordance with Hussain *et al.* [35] who recorded 66.05% carbohydrate in *Luffa actangula*. Franca *et al.* [32] was also observed 63.06% carbohydrate content for cucumber fruit. Crude fat is principle source of energy and play crucial role in transportation of lipid soluble biomolecules, maintain cell structure and various cell metabolism [36]. In present study, we found high amount of crude fat in fruit (11.25 %) than leaf (3 %). On the other hand, Franca *et al.* [32] recorded 5.42% crude fat from the fruit of cucumber. Similarly crude fat content of leaf appeared to be significantly higher than the value of crude fat (0.006%) from four cucurbitaceae species [37]. In contrast, our result is good agreement with Blessing *et al.* [38] who reported the range of fatty acid content (1.15-2.63%) for 10 accessions of *Cucurbita* spp. It has proved that crude fibre play important role to reduce cholesterol level in the blood, risk of cancer, heart diseases, constipation and diabetes [39]. High crude fibre content was recorded in fruit (42.80%) than leaf (17.40%). In present study, crude fibre content of leaf was appeared to be higher than *Cucurbita ficifolia*, *Momordica charantia*, *Trichosanthes cucumerina* and *Luffa cylindrica* (12-13.50%) [37]. Similarly, crude fibre in *C. sativus* f. *hardwickii* fruit seems to be higher than cultivated cucumber (4.60%) [32]. From the result it is cleared that fruits can be served as a good source of crude fibre. The carotenoids is acts as a pro-vitamin and precursor. It is most efficient radical scavenger in biological system and reduces risk of various degenerative diseases like cardiovascular, cataract, cancer and diabetes [40]. The significant amount of carotenoids was recorded in leaf (191.67 mg/100 g DW) while considerable amount of carotenoids was recorded in fruit (118.54 mg/100 g DW). Vitamins are the organic compounds required in trace amount for various biological processes. Vitamins C is a potent antioxidant molecule that prevents various oxidative stress related diseases [41]. Present study showed considerable Vitamin C in fruit (123.98 mg/100 g DW) than leaf (36.16 mg/100 g DW). In addition, Vitamin E was also recorded in leaf (8.83 mg/100 g DW) and fruit (27.25 mg/100 g DW). Hence fruit and leaf of plant are good source Vitamin C and E. Olajire and Azeez [42] had reported significantly least amount of Vitamin C (16.67 mg/g extract) from cucumber than our calculated value. On the other hand Blessing *et al.* [38] also reported Vitamin C content (0.701- 3.473 mg/100 g) from 10 accessions of Pumpkin. Naglaa *et al.* [43] reported significant amount of Vitamin E from seeds (1.23 mg/100g) and fruit pulp

(0.2mg/100g) of *Cucurbita moschata*. Vitamin E is a strong antioxidant biomolecule that protect cells from oxidative damage [44]. By using values of protein, carbohydrates and fats, energy value was calculated for leaf (263.46 Kcal/100g) which was similar to fruit (237.41 Kcal/100g). Our results are comparable with seeds *Benincasa hispida* [45]. On the other hand, dry pulp of *Citrullus lanatus* (365.82 Kcal/100g) and *Cucurbita pepo* (371.21 Kcal/100g) exhibited slight higher caloric values [46]. Similarly, high energy value recorded for seeds of Pumpkin (564 Kcal/100g) and Watermelon (582 Kcal/100 g) [47].

Antinutritional factors (ANFs)

Antinutritional factors (ANFs) are a natural or synthetic compound that reduces nutrient intake, protein digestibility and bioavailability of minerals that leads to drastically decline in nutritional quality and their utilization of biological resources. The well known antinutritional factors in plants are phenolics, phytate, tannins, saponins, lectins and trypsin inhibitor [48]. In our investigations, we noted negligible phytic acid in leaf (0.38 %) and fruit (0.23 %) (Table 1). Lower levels of phytate has been reported in pumpkin fruit (0.002-0.021 mg /100 g) and seeds (0.035 %) [38, 47]. Our results are comparable with Philip and Prema [48]. On the other hand, a high level of phytic acid was recorded for some leguminous plants viz. *Glycine soja* (1.64-2.07%), *Cajanus cajan* (2.0-2.4%),

Vigna unguiculata (2.0-2.9%) [49, 50]. Phytate reduces protein digestibility by inhibiting proteolytic enzymes or interacting with proteins by forming enzyme resistance complex. Like the phytate, tannins and trypsin inhibitor are also interacts with protein or carbohydrate and reduces their digestibility by converting it into enzyme resistant substance [51]. The remarkable tannin content was recorded in leaf (1.78%) and fruit (1.40%) of *C. sativus* f. *hardwickii* (Table 1). The tannin content was very low as compared to leaf (10.29%) and fruit (4.99%) of *Cucumis dipsaceus*. Similar results were obtained in Pumpkin [38]. The critical level of nitrate causes nitrate poisoning by conversion of haemoglobin to methaemoglobin in blood [52]. In present study, significant amount of nitrate was recorded in both leaf (1.28%) and fruit (0.26%) (Table 1). Similarly, Karaye et al. [53] recorded least nitrate content in both *C. maxima* and *C. metuliferus* (8.30, 6.80 mg/100 g) respectively. The ingestion of significant amount of trypsin inhibitors, disrupt the digestive process and may lead to undesirable physiological reactions [54]. In our result, we found high trypsin inhibitor activity in fruit (4.6 TIU/mg proteins) compared to leaf (3.5 TIU/mg proteins) (Table 1). These results are comparable with Chandran et al. [33] and Nivedhini et al. [34]. It was noticed that wild plants content high level of antinutritional factor than cultivated plant but our study exhibited resemblance with cultivated species. Furthermore, they can be reduced by different processing methods dehulling, soaking, cooking and fermenting [51].

Table 1. Proximate composition of leaf and fruit of *C. sativus* f. *hardwickii*.

Proximate analysis	Leaf	Fruit
Moisture %	84.63±0.20	88.97±0.15
Ash %	20.50±0.15	11.90±0.20
Carbohydrate %	53.40±0.20	32.35±0.22
Crude protein %	5.71±0.72	1.77±0.08
Crude fat %	03.00±0.15	11.25±0.16
Crude fibre %	17.40±0.24	42.80±0.26
Energy value (kcal/100g)	263.46± 0.20	237.41±0.17
Vitamin C (mg/100g)	36.16±0.59	122.98±0.97
Vitamin E (mg/100g)	8.83±0.09	27.25±1.08
Carotenoids (mg/100g)	191.67±0.10	118.54±0.23
Phytic acid (%)	0.38±0.001	0.23 ±0.001
Tannins (%)	1.78±0.085	1.40±0.034
Nitrate (%)	1.28±0.021	0.26±0.019
Trypsin inhibitor (TIU/mg protein)	3.50 ±0.03	4.60±0.04

All values are means of triplicate determination expressed on dry weight basis ± standard error.

Phytochemicals assays

Total phenolics content

Phenolics are important group of natural antioxidant found in most of the plant and effectively scavenge free radicals from biological system [55]. It was found that high content of phenolics linearly correlated with high antioxidant activity. In our study, we found that methanol and aqueous extract of leaf exhibited higher phenolic

content (34.08-37.47 mg TAE/g extract) (Table 2). Our results are in good agreement with Fidrianny et al. [56] who reported the remarkable amount of phenolics (40 mg GAE/g of extract) from ethyl acetate leaf extract of *Sechium edule*. In contrast, Nasrin et al. [13] recorded significant amount of phenolics (261.31 mg GAE/g of sample) from leaf extract of cucumber. Chu et al. [57] reported very less amount of phenolics in cucumber (14.37 mg/100 g).



Table 2. Total phenols, total flavonoids, total terpenoids content of leaf and fruit extracts of *C. sativus* f. *hardwickii*.

Antioxidant Assay	Leaf extracts				Fruit extracts			
	Hexane	Chloroform	Methanol	Aqueous	Hexane	Chloroform	Methanol	Aqueous
Total Phenols ^a	2.84±0.07 ^e	2.19±0.46 ^e	34.08±1.69 ^b	37.47±1.26 ^a	0.94±0.08 ^e	1.81±0.20 ^e	28.25±1.06 ^c	10.57±0.10 ^d
Total flavonoids ^b	14.96±1.06 ^d	2.48±0.17 ^g	37.88±1.35 ^a	27.88±0.45 ^b	10.21±0.50 ^e	6.08±0.70 ^f	20.83±0.17 ^c	10.41±1.12 ^e
Total terpenoids ^c	486.73±1.00 ^b	1037.03±1.03 ^a	294.79±1.04 ^d	50.06±0.94 ^g	86.51±0.98 ^f	364.55±0.99 ^c	189.11±0.99 ^e	18.58±0.97 ^h

^a mg tannic acid equivalent/g extract, ^b mg catechin equivalent/g extract, ^c mg ursolic acid/g extract. Values are means of three replicate determinations ± standard error. Values in the same row with different letters showed statistically significant differences ($p < 0.05$) according to Duncan test.

Total flavonoids Content

Flavonoids are most common and diverse group of bioactive metabolites. They are low molecular weight phenolics compounds having effective radical scavenging and metal chelating activity [58]. In present study, leaf extracted with methanol exhibited higher flavonoids content (37.88 mg CE/g extract). Similarly, fruit extracted with methanol showed significant amount of flavonoids (20.83 mg CE/g extract) (Table 2). These results are lined with Akter et al. [59] who reported high amount of flavonoids (823.88 mg quercetin equivalent/g extract) in methanolic aerial part extract of *Trichosanthes dioica*. Similarly, Nivedhini et al. [34] and Fidrianny et al. [56] also reported highest flavonoids content in methanol and hexane extract of *C. dipsaceus* and *M. charantia*.

In vitro antioxidant assays

DPPH radical scavenging activity

In antioxidant studies, DPPH radical scavenging method is used due to its simplicity and stability. The given method is based on reduction of DPPH radical by hydrogen donating antioxidant compounds and subsequently forms of more stable DPPH molecule. Methanolic solution of DPPH radical is appearing a deep violet color which shows a strong absorption at 517 nm and absorption decreases due to DPPH radical scavenged by the antioxidant. The percentage of scavenging of DPPH was contingent on plant part, concentration and solvent used. Concentration of the sample needed to decrease initial concentration of DPPH by 50% (IC_{50}) under the experimental condition was determined. Therefore, lower value of IC_{50} indicates a higher antioxidant activity. In the detailed study, all solvent extracts of fruit except hexane revealed maximum radical scavenging activity than leaf extracts. Among the leaf extracts, hexane described maximum scavenging activity (IC_{50} 337 μ g/mL). Nonetheless, among the fruit extract, methanol (IC_{50} 598 μ g/mL) represented highest DPPH scavenging activity (Figure 1). The results obtained are in accordance with Gill et al. [11] who reported

that methanol extract of cucumber seeds showed significant radical scavenging activity (76.2%) at a concentration of 700 μ g/mL. Nasrin et al. [13] observed significantly high radical scavenging activity (IC_{50} 13.06 μ g/mL) for methanol extract of cucumber. In contrast, Kumar et al. [15] reported noticeable radical scavenging activity (56.15%) for aqueous fruit extract of cucumber at 500 μ g/mL extract.

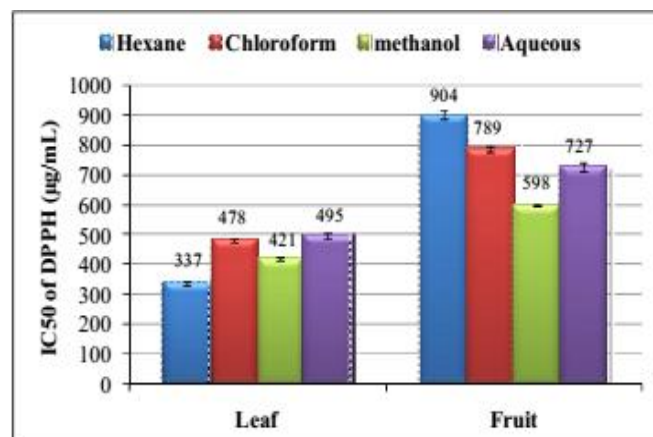


Figure 1. DPPH radical scavenging activity of leaf and fruit extracts of *C. sativus* f. *hardwickii*. Values are means of three replicate determinations ± standard error. Bars having different values represent IC_{50} .

ABTS + Radical Scavenging Activity

ABTS + radical scavenging assay is an excellent tool for determining the antioxidant activity. Assay is mainly based on the reduction of ABTS + radical in the presence of hydrogen donating antioxidant. ABTS radical solution is appearing a green color with maximum absorbance at 734nm and resulting decoloration due to reduction of ABTS + radical by antioxidant. In our study, methanol extract of fruit showed higher radical scavenging capacity (42.11 mg TE/g of extract) compared to other extracts. Similarly, in leaf,

hexane, methanol and aqueous extracts showed comparable radical scavenging activity (19.93, 19.85 and 19.08 mg TE/g extract respectively) (Figure. 2). These results concurred with Nivedhini et al. [34] where methanol extract of *Cucumis dipsaceus* fruit showed higher ABTS + radical scavenging activity (4.90 mg trolox/g extract). However, aqueous leaf extract of *Cucumis dipsaceus* exhibited highest radical scavenging activity than other extract [33].

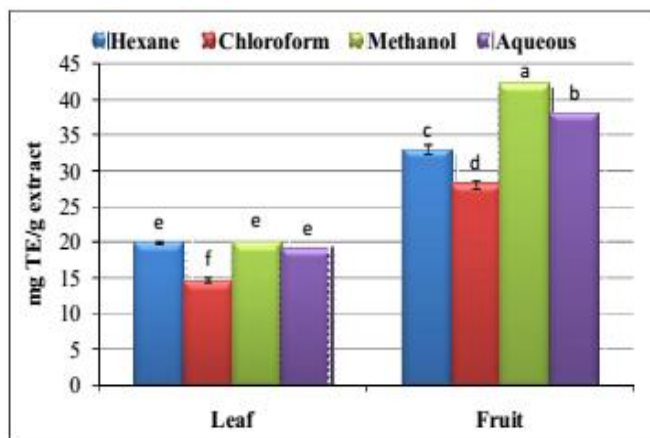


Figure 2. ABTS + radical scavenging activity of leaf and fruit extracts of *C. sativus* f. *hardwickii*. Values are means of three replicate determinations \pm standard error. Bars having different alphabets showed statistically significant differences ($p < 0.05$) according to Duncan multiple range test.

Metal Chelating Activity

The transition metal ion is necessary for many biological processes like respiration, oxygen transport and activation of many enzymes. This metal ion play vital role in various oxidative process such as Haber-Weiss and Fenton-type reactions and resulting formation of various reactive oxygen species. These ROS causes oxidation of lipid, protein, nucleic acid. Hence such metal toxicity reduced by antioxidants which result inhibition of ROS [60]. In present investigation, aqueous extracts of leaf and fruit exhibited significant high antioxidant activity (5.33 and 22.38 mg EE/g extract respectively) compared to other extracts (Figure 3). All fruit extract showed highest metal chelating activity than leaf extracts. These results are lined with Chandran et al. [33] where hot water leaf extract of *Cucumis dipsaceus* exhibited higher chelating activity.

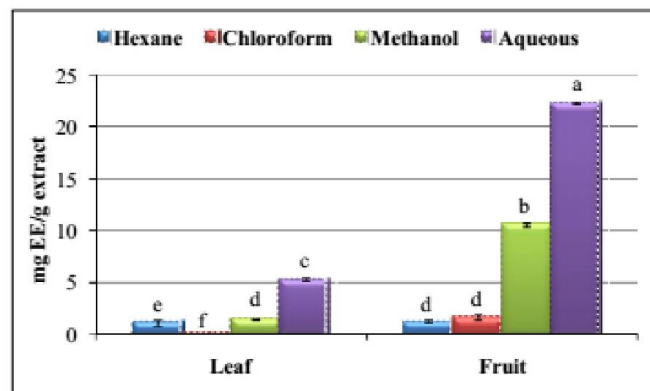


Figure 3. Metal chelating activity of leaf and fruit extracts of *C. sativus* f. *hardwickii*. Values are means of three replicate determinations \pm standard error. Bars having different alphabets showed statistically significant differences ($p < 0.05$) according to Duncan multiple range test.

FRAP Assay

FRAP assay is based on ability of antioxidant compounds to reduce the TPTZ-Fe(III) complex to the intensely blue colored TPTZ-Fe(II) complex in acidic medium with absorption maximum at 593nm. Generally reducing properties are related with presence of reductant compounds that inactivate the oxidant by donating electron called as redox reaction. The results indicated that, among leaf extracts, water showed significantly higher FRAP value (280.87 mg Fe (II)/g extract) compared to other extract (Figure 4). However, in fruit, methanol extract recorded higher FRAP value (313.38 mg Fe (II)/g extract). Similarly, methanolic fruit extract showed appreciable FRAP value (276.58 μ M Fe (II)/mg extract) [34].

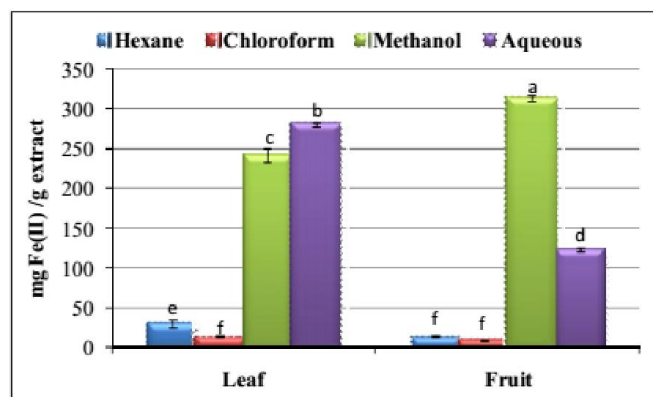


Figure 4. FRAP activity of leaf and fruit extracts of *C. sativus* f. *hardwickii*. Values are means of three replicate determinations \pm standard error. Bars having different alphabets showed statistically significant differences ($p < 0.05$) according to Duncan multiple range test.

Phosphomolybdenum Assay

The antioxidant capacities of different extracts were evaluated and results are depicted in Figure 5. It is based on reduction of Mo (VI to Mo V) by extract and subsequent formation of green color phosphate-Mo (V) complex that shows maximum absorption at 695nm. Methanolic extract of leaf and fruit extract showed maximum antioxidant activity (159.40, 348.04 mg AAE/g extract) than other solvent tested (Figure 5). However, methanolic aerial part extract of *Trichosanthes dioica* showed significant antioxidant activity (605.92 mg AAE/g of extract) as reported by Akter *et al.* [59]. In contrast, Chandran *et al.* [33] studied that aqueous leaf extract of *Cucumis dipsaceus* exhibited remarkable total antioxidant activity (238.8 mg AAE/g of extract).

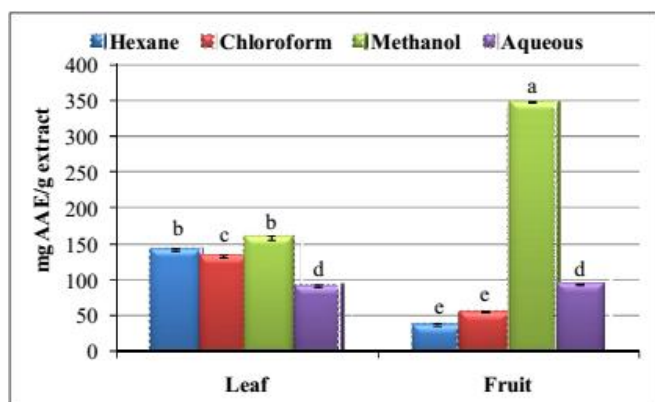


Figure 5. Phosphomolybdenum reduction assay of leaf and fruit extracts of *C. sativus* f. *hardwickii*. Values are the means of three replicates \pm standard error. Values are means of three replicate determinations \pm standard error (SE). Bars having different alphabets showed statistically significant differences ($p < 0.05$) according to Duncan multiple range test.

Qualitative and Quantitative Analysis of Phenolic Compounds

Phenolics were identified and quantified from methanol extract of leaf and fruit are shown in Table 3. Phenolic compounds such as tannic acid, gallic acid, hydroxybenzoic acid, vanillic acid, coumaric acid were analysed by RP-HPLC. The chromatograms of methanol extract of leaf and fruit are shown in Figure 6 and 7. Our results revealed that tannic acid was major phenolic present in both leaf and fruit (9.170, 2.580 mg/g DW respectively) than other phenolic compounds. However gallic acid and hydroxybenzoic acid is present in moderate concentration. Among the phenolic compounds, vanillic acid (0.389 mg/g DW) was detected only in leaf. Similarly, coumaric acid (0.108 mg/g DW) is recorded exclusively in fruit. Several studies reported that tannic acid is potent free radical scavenger and exhibited antimutagenic and anticarcinogenic properties. It is also use as food additives [61,62]. Likewise gallic acid was found to be effective in breast cancer and diabetes [63,64]. Ghanbari *et al.* [65] reported that coumaric acid, vanillic acid, hydroxybenzoic acids are good antioxidants having several health benefits. In present study, gallic acid content of leaf and fruit were appeared to be higher than *Benincasa hispida* [64]. Our results are lined with Venkataramaiah and Rao [66] where hydroxybenzoic acid, p-coumaric acid and vanillic acid were reported from twelve members of Cucurbitaceae. The various investigations have proved that phenolics compounds are directly associated with the antioxidant activity and which has diverse health benefits regarding prevention of cardiovascular disease, cancer and reduction of oxidative stress related diseases [55, 67]. Based on the results, highly polar solvents such as aqueous and methanol extracts exhibited higher antioxidant potential associated with phenolics. All phenolics compounds identified in this study are a potent free radical scavenger as well as effective in prevention of several diseases.

Table 3. Phenolic compounds in the methanol extracts of leaf and fruit extracts of *C. sativus* f. *hardwickii*.

Sr. No.	Phenolic compounds	Retention time	Leaf (mg/g dry weight)	Fruit (mg/g dry weight)
1.	Tannic acid	1.068	9.170 \pm 0.13	2.580 \pm 0.08
2.	Gallic acid	1.374	0.626 \pm 0.11	0.417 \pm 0.03
3.	Hydroxybenzoic acid	3.259	0.436 \pm 0.05	0.352 \pm 0.00
4.	Vanillic acid	4.066	0.389 \pm 0.00	-
5.	Coumaric acid	7.094	-	0.108 \pm 0.09

Values are means of three replicate determinations \pm standard error.

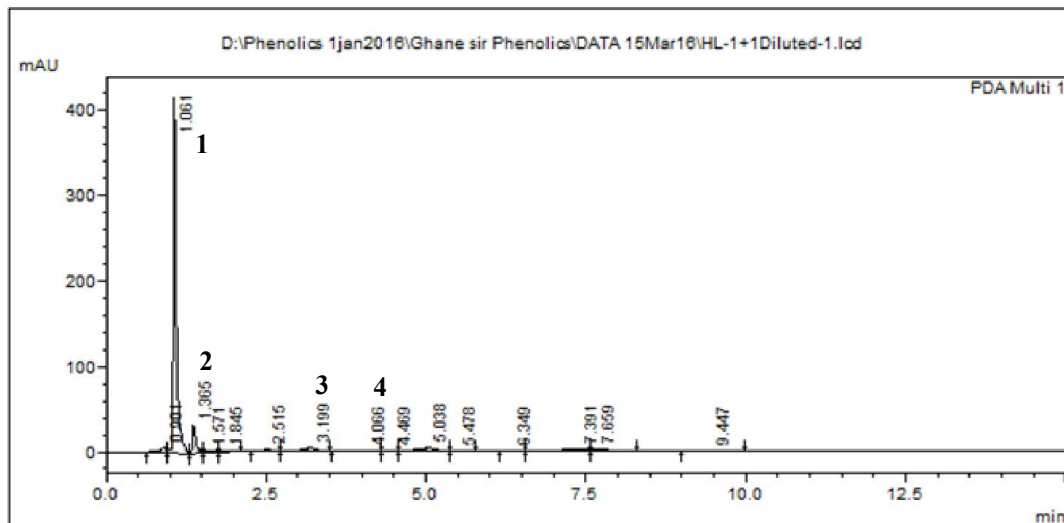


Figure 6. HPLC chromatogram of leaf extract of *C. sativus* f. *hardwickii* where the peaks were identified as follows: 1. tannic acid 2. gallic acid, 3. hydroxybenzoic acid, 4. vanillic acid.

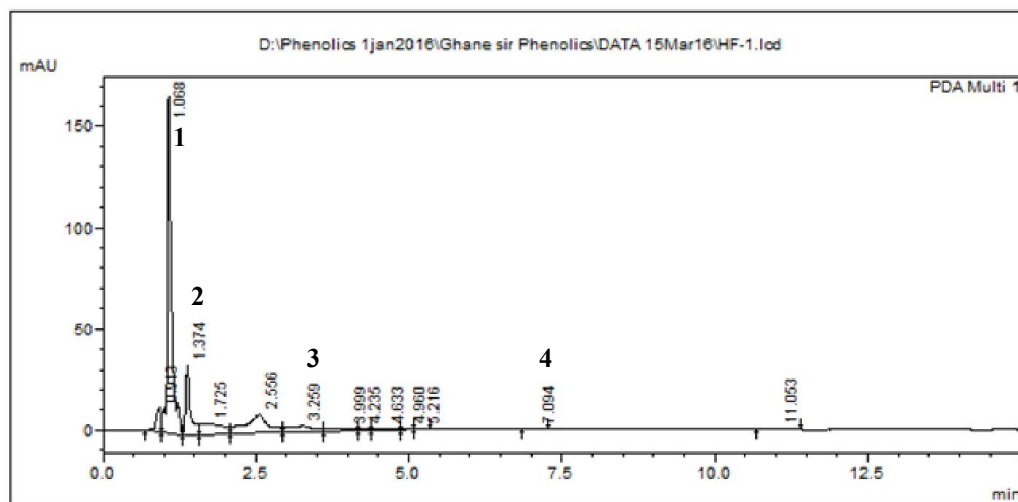


Figure 7. HPLC chromatogram of fruit extract of *C. sativus* f. *hardwickii* where the peaks were identified as follows: 1. tannic acid 2. gallic acid, 3. hydroxybenzoic acid, 4. coumaric acid.

Conclusion

The nutrition, anti-nutrition, potential antioxidant activities and phenolics profiling using RP-HPLC of wild progenitor of the Cucumber i. e. *Cucumis sativus* forma *hardwickii* were assessed for the first time. The high nutritive value and less antinutritional factor suggest that it is a promising source of food. Further, phytochemicals present in the leaf and fruit suggest that the antioxidant activities of this unexplored plant can be mainly due to the phenolic and flavanoids. These findings confirm the antioxidant potential and health-promoting properties of this neglected cucurbit and may provide a vital source of food, natural antioxidant and other pharmaceutically important compounds.

Author's contributions

U.A.A. and S.G.G. contributed equally in collection of plant material, experiment design, performing experiment, data compilation and statistical analysis. Both the authors wrote, read and approved the final manuscript.

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