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Nutraceutical and antioxidant properties of *Cucumis hardwickii* Royle: A potent wild edible fruit collected from Uttarakhand, India

Tapan Seal, Kausik Chaudhuri and Basundhara Pillai

Abstract
The objective of the present study was to evaluate the proximate composition, mineral content, simultaneous quantitation of water-soluble vitamins like ascorbic acid (C), thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6) and folic acid (B9) by HPLC of the fruits of a wild edible fruit, *Cucumis hardwickii* Royle The *in vitro* antioxidant properties and HPLC fingerprinting assay for the simultaneous estimation of phenolic acids and flavonoids of the 80 % aqueous ethanol extract of this fruit has also carried out. The fresh fruits of *C. hardwickii* were collected from Uttarakhand, India the proximate parameters, minerals and toxic heavy metals such as lead, cadmium, chromium and mercury were evaluated in the wild edible fruit using standard food analysis techniques. The 80% aqueous (aq.) ethanol extract of the plant was examined for its antioxidant activities. The water soluble vitamins, phenolic acids and flavonoids were estimated by HPLC using Acclaim C 18 column (5 µm particle size, 250 x 4.6 mm), Dionex Ultimate 3000 liquid chromatograph and detection was carried out in photo diode array (PDA) detector. The present study showed the presence of very good amount of protein (11.9±1.36%), carbohydrate (12.57±1.65%) and minerals in various amount. The heavy metals Pb and Cr were detected in very low amount and Cd and Hg were not detected in this fruit. The total phenolic and flavonoid content were found 540.12 ± 1.72 mg/100gm and 74.34 mg/100gm respectively in the plant. The plant contains a very good amount of vitamin C (130.76±0.05 mg/100gm) along with water soluble B vitamins ranged between 0.27 to 1.61 mg/100gm. The HPLC analysis also showed the presence of phenolic acids and flavonoids in various amounts in this wild edible plant. The outcome of investigation indicates that proximate composition, mineral contents and water soluble vitamin content of this fruit under investigation were richer than that of the commercial vegetables and could be used for the nutritional purpose. This fruit is also a good source of natural antioxidants and has beneficial effect to human being. The present study also gives an account of traditional significance of the wild plants under investigation.

Keywords: *Cucumis hardwickii*; Nutritive value; Vitamin content by HPLC, Antioxidant; Phenolic acids & flavonoids by HPLC.

1. Introduction
Cucumis hardwickii (Royle), belonging to the family Cucurbitaceae, are reported in the higher elevations in the Himalayan foot hill of India [1] and distributed in different states of India i.e. Himachal Pradesh, Uttar Pradesh, Uttarakhand, Rajasthan, Madhya Pradesh, Chhattisgarh, Odisha and Western ghats of Maharashtra. The family is widely grown for its edible fruits in nearly all the tropical, semitropical and temperate regions of the world. Ethnobotanical explorations revealed that this plant is used for the treatment of diabetes [2] and intestinal worms [3].

The plants of the family Cucurbitaceae are well known for having several essential nutrients, minerals, vitamins, dietary fibres and a number of nutraceuticals and phytomedicinal compounds. The tender fruits of *Cucumis* spp. are eaten as a vegetable directly in the form of salad, fried and pickled. The flesh of cucumber is mixed with grinded urad dal and mung dal for preparing (cucumber badi) for using as a dal or vegetable. Some wild cucurbits like *Citrullus colosynthus, Cucumis hardwickii* grows naturally around the household and are utilized for the treatment of different human ailments [4].

The wild edible fruits have been recognized to have rich nutritional value and play an important role to the rural poor and tribal communities in the form of food and nutrient supplement. Recently, a lot of interest has been focused to evaluate various wild edible fruits because they serve as an indispensable constituent of human diet replenishing the body with minerals, vitamins and certain hormone precursors, in addition to protein and energy.

Due to the presence of protein, carbohydrate and various macro nutrients, the wild edible fruits play an important role to reduce the risk of various diseases like cancer, coronary heart attack, diabetes, etc. The wild vegetables have become a commercial crop nowadays with increasing market potential due to the absence of residues from pesticides or fertilizers[5].

The wild edible fruits provide an abundance of vitamins to meet one's nutritional needs. They are essential for metabolism because of their redox chemistry and role as enzymatic cofactors, not only in animals but also in plants. Even though vitamins are required in small amounts daily in our diet, they play a vital role in our health. The consumption of leafy vegetables and fruits rich in vitamins, are reported to reduce the risk of attack of various acute and chronic diseases [6].

The wild edible fruits, herbs, vegetables, cereals and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of the food. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers and consumers whose ultimate aim is to discover the functional food with specific health effects [7]. The antioxidant activities of the plants represent an important parameter to evaluate their biological importance. Therefore, it is necessary to characterize and quantify the important compounds present in the plant and also to validate the method of separation and identification of active constituents. The use of these plants in folk medicine and its nutraceutical role provide unequivocal testimony to the fact. The extraction of polyphenolic compounds from plant is highly dependent on the polarity of the solvent because polar compound is easily extracted using polar solvent. Thus, the solvent used for the extraction of bioactive compounds must be critically chosen because it will influence the quantity and quality of the final extract [8]. There is no information available in the literature on nutritional composition, mineral content, and antioxidant properties of *C. hardwickii* fruit. Therefore, the aim of this study is to determine the nutritional composition, mineral content, vitamin content, antioxidant properties of the fruits along with quantification of phenolics and flavonoids using HPLC in order to establish its nutraceutical properties as well as the result of investigation will encourage the common people for domestication.

2. Materials and Methods

2.1 Collection and identification of plant

Fig 1: Fruits of *Cucumis hardwickii*

The fresh fruits of *Cucumis hardwickii* Royle were collected (Fig. 1) from Dehradun, Uttarakhand and the identification was authenticated by the Botanical Survey of India, Howrah. The voucher specimens were preserved at the Plant Chemistry department of our office. The fruits were taken in our laboratory at refrigerated temperature using cold packs. The refrigerated plant samples were stored at -15^{0}C and one part processed for vitamin estimation. The other parts were shed-dried, pulverized and stored in an airtight container to evaluate proximate composition, minerals content and antioxidant properties.

2.2 Chemicals

The standards chemicals like ascorbic acid (C), thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), folic acid (B9), phenolic acids (gallic acid, protocatechuic acid, gentisic acid, chlorogenic acid, *p*-hydroxy benzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid, salicylic acid and ellagic acid), flavonoids (catechin, rutin, myricetin, quercetin, naringin, apigenin and kaempferol), 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA), Folin-Ciocalteus's phenol reagent, potassium ferricyanide, potassium per sulphate, Aluminium chloride, $FeCl_3$, anthrone and sodium carbonate and the HPLC-grade solvents such as acetonitrile, methanol, water sodium dihydrogen phosphate and trifluoroacetic acid were purchased from Merck (Germany). All the chemicals used including the solvents, were of analytical grade.

2.3 HPLC equipment

HPLC analyses were performed using Dionex Ultimate 3000 liquid chromatograph including a diode array detector (DAD) with 5 cm flow cell and with Chromeleon system manager as data processor. Separation was achieved by a reversed-phase Acclaim C18 column (5 micron particle size, 250 x 4.6 mm). 20 μL of sample was introduced into the HPLC column.

2.4 Estimation of ash content

Five gm of fruits were weighed in a silica crucible and heated in the muffle furnace for about 5-6 h at 500 °C. It was cooled in a desiccator and weighed. It was heated again in the furnace for half an hour, cooled and weighed. This was repeated consequently, until the weight became constant (ash became white or grayish white). Weight of ash gave the ash content [9].

Ash content (%) = Weight of ash × 100/Weight of sample.

2.5 Estimation of moisture content

Two gm of fresh fruits were taken in a flat-bottom dish and kept overnight in an air oven at 100–110°C and weighed. The loss in weight was regarded as a measure of moisture content in the sample [9].

Moisture (%) = [(Weight of original sample−Weight of dried sample)] x 100 /
Weight of original sample

2.6 Estimation of crude fat content

Two gm moisture free of fruits were extracted with petroleum ether (40-60°C) in a Soxhlet apparatus for about 6-8 h. After boiling with petrol, the residual petrol was filtered using Whatman no. 40 filter paper and the filtrate were evaporated in a pre-weighed beaker. Increase in weight of a beaker gave crude fat. Percentage of fat content was calculated using the following formula [9].

Crude fat (%) = Weight of fat in sample × 100/Weight of dry sample.

2.7 Estimation of crude fibre content

Two gm of moisture and fat-free material of fruits were treated with 200 mL of 1.25 % H_2SO4. After filtration and washing, the residue was treated with 1.25 % NaOH. It was then filtered, and residue was washed in boiling water followed by 1 % HNO_3 and again with hot water. The washed residue was dried in an oven at 130 °C to constant weight and cooled in a desiccator. The residue was scraped into a pre-weighed porcelain crucible, weighed, heated in muffle furnace at 550 °C for two hours, cooled in a desiccator and reweighed. Crude fibre content was expressed as percentage loss in weight on ignition [9].

Crude fibre (%) = (Weight of residue − Weight of ash) x 100/ Weight of the sample

2.8 Estimation of crude protein content

The crude protein was determined using micro Kjeldahl method. Two gm of fruits sample were decomposed by digestion with concentrated sulphuric acid in the presence of a catalyst, until the mixture was clear. The digest was taken in a round-bottom flask, and the solution was diluted with distilled water. An excess of sodium hydroxide solution (40%) was added to the diluted reaction mixture, the liberated ammonia was distilled in steam and absorbed in 25 ml N/20 sulphuric acid. Titration of the residual mineral acid with standard sodium hydroxide gives the equivalent of ammonia obtained from the weight in the sample taken. From this, the percentage of nitrogen in the compound was calculated. Based on early determinations, the average nitrogen (N) content of proteins was found to be about 16 percent, which led to use of the calculation N x 6.25 (1/0.16 = 6.25) to convert nitrogen content into protein content [9].

2.9 Estimation of carbohydrate content

100 mg of fruits were hydrolysed with 5 ml 2.5 N HCl, cooled to room temperature and neutralised with solid sodium carbonate until the effervescence ceases. The solution filtered in a 100 ml volumetric flask and make up the volume with distilled water. To one ml of this solution, 4 ml freshly prepared anthrone reagent (200 mg anthrone dissolved in 100 mL of ice-cold 95% H_2SO_4) were added and heated in a water bath for eight minutes. The mixture was cooled rapidly, a dark green colour appeared and the absorption at 630 nm was measured (UV-visible spectrophotometer Shimadzu UV 1800). The total carbohydrate content was expressed as glucose equivalents using the following equation based on the calibration curve $y = 0.0081x + 0.2475$, $R^2 = 0.9993$ where y was the absorbance and x concentration of glucose in mg/ml [10].

2.10 Estimation of energy content

The three components of foods which provide energy are protein, carbohydrate and fat. One gram carbohydrate and protein each yield four kcal energy whereas one gram fat yields nine kcal energy. Therefore, the energy (kcal/100gm) contents of each plant sample were determined by multiplying the values obtained for protein, fat and available carbohydrate by 4.00, 9.00 and 4.00, respectively and adding up the values [9, 11].

2.11 Estimation of minerals in plant material

One gram of fruits were taken in a precleaned and constantly weighed silica crucible and heated in a muffle furnace at 400°C till there was no evolution of smoke. The crucible was cooled at room temperature in a desiccator and carbon-free ash was moistened with concentrated sulphuric acid and heated on a heating mantle till fumes of sulphuric acid ceased to evolve. The crucible with sulphated ash was then heated in a muffle furnace at 600°C till the weight of the content was constant (~2–3 h). One gram of sulphated ash obtained above was dissolved in 100 mL of 5 % HCl to obtain the solution ready for determination of mineral elements through atomic absorption spectroscopy (AAS) (AA 800, Perkin-Elmer Germany). Standard solution of each element was prepared and calibration curves were drawn for each element using AAS [12]. All assays were carried out in triplicate and values were obtained by calculating the average of three experiments and data are presented as Mean ± SEM.

2.12 Quantification of water soluble vitamin by HPLC

2.12.1 Preparation of mixture standard vitamin solutions

The stock standard solutions of vitamin C, B1, B3, B5 and B6 and were prepared by dissolving 25 mg of the each standard in one ml 0.1 M hydrochloric acid in 25 ml standard volumetric flask and topped up to mark with double distilled water. For preparation of standard stock solutions of vitamin B9 and B2, 25 mg of the each standard were dissolved in one ml 0.1 M sodium hydroxide in 25 ml standard volumetric flask and made up to mark with double distilled water. The standard solution was stored in amber-glass bottles in the refrigerator at 4^0C. The working standards were prepared from the stock standard solutions by mixing 100 µl mixed vitamins standard (vitamin B9, B5 and B2), 800 µl phosphate buffer (1 M, pH 5.5) and 100 µl mixed vitamins standard (vitamin C, B1, B6 and B3) which represent 100 µg/ml mixed working standards. The working standard solutions of concentrations 20, 40, 60 and 80 µg/ml were prepared accordingly.

2.12.2 Preparation of sample solution

Plant materials were cleaned and the inedible portions were removed. The edible parts were rinsed thoroughly with tap water and then with distilled water. The washed plant materials were dried with clean cloth, were cut into very small pieces, frozen in liquid nitrogen, freeze-dried and kept at -20 ^{0} C until analysis.

One gm of freeze-dried fruits was soaked in 10 ml water. Then 1 ml 0.1 M and 10 ml phosphate buffer (1M, pH 5.5) were added to it and kept in dark for 24 hours. The solution was first filtered through a Whatman No. 1 filter paper and the resulting filtrate was taken in a 25 ml volumetric flask and solution was topped up to the mark with HPLC grade water. The sample solution was filtered through 0.45 μm membrane filter before injection into LC system. The stock solutions of sample were kept in a refrigerator for further use [13].

2.12.3 Chromatographic analysis of water soluble vitamins

The chromatographic analysis was carried out following the method as described by *Marco Ciulua* [13] with minor modification. The mobile phase contains acetonitrile (Solvent A) and aqueous trifluoro acetic acid (TFA, 0.01% v/v) (Solvent B), the column was thermostatically controlled at 22^0 C and the injection volume was kept at 20 µl. A gradient elution was performed by varying the proportion of solvent A to solvent B. The gradient elusion was 1 % A and 99 % B with flow rate 0.5 ml/min in 5 min, from 1 % to 25% A with flow rate 0.5 ml/min for 16 min, 45 % A, with flow rate 0.5 ml/min for 8 min. from 45 to 1 % A with flow rate 0.5 ml/min in 5 min. The mobile phase composition back to initial

condition (solvent A: solvent B: 1: 99) in 34 min and allowed to run for another 1 min, before the injection of another sample. Total analysis time per sample was 35 min.

The various concentrations of (20, 40. 60, 80 and 100 µg/ml) vitamin working standards were injected into the HPLC column separately and the retention times were noted and used to identify the vitamins in the sample.

HPLC Chromatograms of all vitamins were detected using a photo diode array UV detector at four different wavelengths (210, 245, 275 and 290 nm) according to absorption maxima of analysed compounds. Each compound in the plant extracts were identified by its retention time and by spiking with standards under the same conditions.

The quantification of the vitamins was carried out by the measurement of the integrated peak area and the content was calculated using the calibration curve by plotting peak area against concentration of the respective standard sample. The data were reported as means ± standard error means of three independent analyses and the method was validated according to the USP and ICH guidelines [14-15].

2.13 Antioxidant activities of *C. hardwickii*

2.13.1 Extraction of plant material

One gram of fruits were extracted with 20 ml 80 % aq. ethanol with agitation for 18 -24 h at ambient temperature. The extract was filtered and diluted to 50 ml and aliquot was analyzed for their total phenolic, flavonoid and flavonol content, reducing power and free radical scavenging capacity.

2.13.2 Estimation of total phenolic content

The amount of total phenolic content of crude extract of *C. hardwickii* was determined according to Folin-Ciocalteu method [16]. 20 - 100 µl of the tested samples were introduced into test tubes. 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured (UV-visible spectrophotometer Shimadzu UV 1800). The total phenolic content was expressed as gallic acid equivalents (GAE) in miligram per gram (mg/g) of extract using the following equation based on the calibration curve y = 0.0013x + 0.0498, R^2 = 0.999 where y was the absorbance and x was the Gallic acid equivalent (mg/g).

2.13.3 Estimation of total flavonoids

Total flavonoids were estimated in *C. hardwickii* using the method of Ordonez *et al*., 2006 [17]. To 0.5 ml of extract, 0.5 ml of 2% $AlCl_3$ ethanol solution was added. After one hour, at room temperature, the absorbance was measured at 420 nm (UV-visible spectrophotometer Shimadzu UV 1800). A yellow color indicated the presence of flavonoids. Total flavonoid contents were calculated as rutin (mg/g) using the following equation based on the calibration curve: y = 0.0182x - 0.0222, R^2 = 0.9962, where y was the absorbance and x was the Rutin equivalent (mg/g).

2.13.4 Estimation of total flavonols

Total flavonols in the fruit extracts were estimated using the method of Kumaran and Karunakaran, 2006 [18]. To 2.0 ml of extract, 2.0 ml of 2% $AlCl_3$ ethanol and 3.0 ml (50 g/L) sodium acetate solutions were added. The absorption at 440 nm (UV-visible spectrophotometer Shimadzu UV 1800) was read after 2.5 h at 20°C. Total flavonol content was calculated as quercetin (mg/g) using the following equation based on the calibration curve: y = 0.0049x + 0.0047, R^2 = 0.9935, where y was the absorbance and x was the quercetin equivalent (mg/g).

2.13.5 Measurement of reducing power

The ability of the extracts to reduce iron (III) was assessed by the method of Oyaizu, 1986 [19]. Extracts (100 µl) of plant extracts were mixed with phosphate buffer (2.5 ml, 0.2 M, *p*H 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 min. Aliquots of 10% trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm. Reducing power is given in ascorbic acid equivalent (AAE) in milligram per gram (mg/g) of dry material using the following equation based on the calibration curve: y = 0.0023x - 0.0063, R^2 = 0.9955 where y was the absorbance and x was the ascorbic acid equivalent (mg/g).

2.13.6 Determination of DPPH free radical scavenging activity

The free radical scavenging activity of the plant samples and butylated hydroxyl toluene (BHT) as positive control was determined using the stable radical DPPH (1, 1-diphenyl-2-picrylhydrazyl) [20]. Aliquots (20 - 100 µl) of the tested sample were placed in test tubes and 3.9 ml of freshly prepared DPPH solution (25 mg L^{-1}) in methanol was added in each test tube and mixed. 30 min later, the absorbance was measured at 517 nm (UV-visible spectrophotometer, Shimadzu UV 1800). The capability to scavenge the DPPH radical was calculated, using the following equation:

DPPH scavenged (%) = {(Ac – At)/Ac} x 100

Where Ac is the absorbance of the control reaction and at is the absorbance in presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration in mg of dry material per ml (mg / ml) that inhibits the formation of DPPH radicals by 50%. Each value was determined from regression equation.

2.13.7 Scavenging activity of ABTS radical cation

The 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation ($ABTS^{\cdot+}$)-scavenging activity was measured according to the method described by Miller[21]. ABTS was dissolved in water to a 7 mM concentration. The ABTS radicals were produced by adding 2.45 mM potassium persulphate (final concentration).The completion of radical generation was obtained in the dark at room temperature for 12–16 h. This solution was then diluted with ethanol to adjust its absorbance at 734 nm to 0.70 ± 0.02. To determine the scavenging activity, 1 ml of diluted $ABTS^{\cdot+}$ solution was added to 10 µl of plant extract (or water for the control), and the absorbance at 734 nm was measured 6 min after the initial mixing, using ethanol as the blank. The percentage of inhibition was calculated by the equation:

ABTS scavenged (%) = $(A_{cont} - A_{test}) / A_{cont} \times 100$

Where A_c and A_s are the absorbencies of the control and of the test sample, respectively. From a plot of concentration against % inhibition, a linear regression analysis was performed to determine the IC_{50} value of the sample.

Values are presented as mean ± standard error mean of three replicates. The total phenolic content, flavonoid content, flavonol content, reducing power and IC_{50} value of plant material was calculated by using Linear Regression analysis.

2.14 Quantification of phenolic acids and flavonoids in the 80 % aq. ethanol extract of *C. hardwickii* by HPLC

2.14.1 Preparation of standard solutions

The stock solution of concentration 1mg / ml was prepared by dissolving 10 mg gallic acid in 1 ml HPLC-grade methanol followed by sonication for 10 min and the resulting volume was made up to 10 ml with the solvent for the Mobile phase (methanol and 0.5% aq. acetic acid 1:9). The same method was followed to prepare the standard stock solutions of the phenolic acids and the flavonoids *viz*. protocatechuic acid, gentisic acid, chlorogenic acid, *p*-hydroxy benzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid, salicylic acid and ellagic acid, catechin, rutin, myricetin, quercetin, naringin, apigenin and kaempferol. The working standard solutions of concentrations 20, 40, 60, 80 and 100 µg/ml were prepared by further dilution of the standard solution with the mobile phase solvent system. The standard and working solutions were filtered through 0.45 µm PVDF-syringe filter and the mobile phase was degassed before the injection of the solutions.

2.14.2 Chromatography analysis for quantification of phenolic acids and flavonoids

HPLC analyses for the quantification of phenolic acids and flavonoids in the fruit extract was performed using Dionex Ultimate 3000 liquid chromatograph including a diode array detector (DAD) with 5 cm flow cell and with Chromeleon system manager as data processor. Separation was achieved by a reversed phase Acclaim C18 column (5 micron particle size, 250 x 4.6 mm). 20 μL of sample was introduced into the HPLC column. The method was validated according to the USP and ICH guidelines [14-15]. The mobile phase contains methanol (Solvent A) and 0.5% aq. acetic acid solution (Solvent B) and the column was thermostatically controlled at 25 ^{0}C and the injection volume was kept at 20 µl. A gradient elution was performed by varying the proportion of solvent A to solvent B. The gradient elusion was 10 % A and 90% B with flow rate 1ml/min to 0.7 ml/min in 27 min, from 10 to 40 % A with flow rate 0.7 ml/min for 23 min, 40% A and 60% B with flow rate 0.7 ml/min initially for 2 min and then flow rate changed from 0.7 to 0.3 ml/min in 65min, from 40 to 44% A with flow rate 0.3 to 0.7ml/min in 70 min, 44% A with flow rate 0.7 to 1ml/min for 10 min duration, solvent A changed from 44% to 58 % with flow rate 1ml/min for 5 min, 58 to 70% A in 98 min at constant flow rate 1 ml/min. The mobile phase composition back to initial condition (solvent A: solvent B: 10: 90) in 101 min and allowed to run for another 4 min, before the injection of another sample. Total analysis time per sample was 105 min.

HPLC chromatograms were detected using a photo diode array UV detector at three different wavelengths (272, 280 and 310 nm) according to absorption maxima of analysed compounds. Each compound was identified by its retention time and by spiking with standards under the same conditions. The quantification of phenolic acids and flavonoids in the fruit extracts were carried out by the measurement of the integrated peak area and the contents were calculated using the calibration curve by plotting peak area against concentration of the respective standard sample. The data were reported with convergence limit in triplicate.

2.15 Statistical analysis

The significant and non-significant variations within water soluble vitamin contents and the ten wild edible plants were analyzed using one-way analysis of variance (ANOVAs). Values are means of five replicates from two experiments, and the presented mean values were separated using Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

3. Results

3.1. Proximate composition of *C. hardwikii*

The fresh fruits of *C. hardwikii* collected from Dehradun were taken for the analysis of proximate composition. The proximate composition of these plants has been presented in Table 1.

Table 1: Proximate composition, Minerals and Vitamin content in the fruits of *C. hardwickii*

Proximate composition	Amount	Minerals	Amount (mg/g)	Vitamin	Amount mg/100gm dry plant material
Ash (%)	16.87 ± 1.35	Sodium (Na)	0.585 **± 0.53**	Vitamin C	130.56±0.20
Moisture (%)	92.58± 2.35	Potassium (K)	36.6 ±1.65	Vitamin B1	1.34±0.05
Protein (%)	11.9 ± 1.36	Calcium (Ca)	28.0 ±1.54	Vitamin B2	0.65±0.03
Fat (%)	9.4 ± 0.36	Copper (Cu)	0.0017 ±0.0002	Vitamin B3	0.28±0.01
Carbohydrate (%)	29.67 ± 1.65	Zinc (Zn)	0.026 ±0.0003	Vitamin B5	0.42±0.01
Crude fibre (%)	20.53 ± 1.55	Magnesium (Mg)	0.55 ±0.12	Vitamin B6	0.93±0.02
Energy (kcal/100gm)	250.88 ± 2.65	Iron (Fe)	0.229 ±0.25	Vitamin B9	1.62±0.01
		Manganese (Mn)	0.0127 ±0.0005		
		Lead (Pb)	0.00017±0.001		
		Chromium (Cr)	0.0012 ± 0.024		
		Cadmium (Cd)	ND		
		Mercury (Hg)	ND		

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

The proximate analysis of *C. hardwikii* fruits revealed ash content 16.87 ± 1.35 gm per 100gm dry sample and moisture content 92.58± 2.35 gm per 100 gm fresh fruit. The fruits were found to contain protein, crude fat, crude fibre and carbohydrate 11.9 ± 1.36%, 9.4 ± 0.36%, 20.53 ± 1.55% and 29.67 ± 1.65% respectively. The energy content of the fruit was found to be 250.88 ± 2.65 kcal/100gm.

3.2. Minerals content in *C. hardwikii*

Table 1 shows the minerals content in mg per gm of the air-dried fruit. Sodium content of the fruit was found to be 0.585 ± 0.53 mg/gm. The fruits was found to be rich in minerals like potassium (36.6 ±1.65 mg/gm), calcium (28.0 ±1.54 mg/gm), magnesium (0.55 ±0.12 mg/gm) and iron (0.229± 0.25 mg/gm) respectively. An appreciable amount of copper, zinc and manganese were also detected in the fruits of *C. hardwickii*. The amount of lead and chromium were noticed in the fruits were 0.00017±0.001 and 0.0012 ± 0.024 mg/gm

respectively whereas other toxic metals like cadmium and mercury were not found in this fruits.

3.3. Identification and quantification of water soluble vitamins in the wild edible fruits by HPLC

A typical HPLC chromatogram of the all standard vitamin mixture recorded at 210 nm is presented in fig. 2.

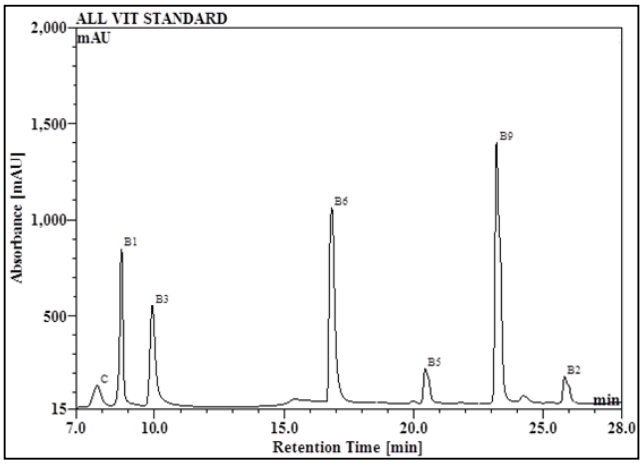

Fig 2: HPLC Chromatogram of mixture of Standard vitamin (C) Ascorbic acid ; (B1) Thiamine ; (B3) Niacin ; (B6) Pyridoxine ; (B5) Pantothenic acid ; (B9) Folic acid ; (B2) Riboflavin

The HPLC method was successfully performed for the estimation of water soluble vitamin e.g ascorbic acid (C), thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6) and folic acid (B9). The quantity of all vitamins of in *C. hardwickii* has been expressed as mg/100gm dry plant material and data presented in table 1.

The HPLC chromatogram of the fruits *C. hardwickii* (Fig.3.) showed the presence of vitamin C (130.56±0.29mg/100gm), B1 (1.34±0.05 mg/100 gm), B2 (0.65±0.03 mg/100gm), B3 0.28±0.01 mg/100gm) B5 (0.42±0.01 mg/100 gm), B6 (0.93±0.02 mg/100 gm) and B9 (1.62±0.01 mg/100gm).

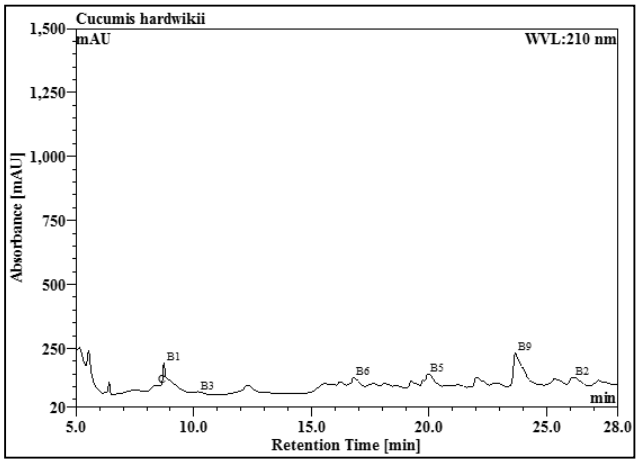

Fig 3: HPLC chromatogram of fruits of *C. hardwickii* showing water soluble vitamins

3.4. Antioxidant activities of the fruits of *C. hardwickii*

In this study, estimation of total phenolic, total flavonoid & total flavonol content, reducing power, ABTS and DPPH methods were employed to evaluate the *in vitro* antioxidant activities of the 80 % aq. ethanol extract fruits of *C. hardwickii* and result has been presented in table 2.

Table 2: Antioxidant properties of the fruits of *C. hardwikii*

Parameters	Amount mg/gm dry extract
Total phenolic content (Gallic acid equivalent)	51.44±1.72
Total flavonoid content (Rutin equivalent)	7.08± 0.07
Total flavonol content (Quercetin equivalent)	10.88±0.05
Reducing power (Ascorbic acid equivalent	10.65±0.30
DPPH Radical scavenging activity (IC_{50})	0.76±0.03
ABTS Radical scavenging activity (IC_{50})	0.27±0.003

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

The result showed total phenolic content in the fruits was 51.44±1.72 mg GAE/gm dry extract. The flavonoid and flavonol amount were detected in the fruits were 7.08±0.07 mg /gm and 10.88±0.05 mg/gm dry extract respectively. The reducing power (AAE) of the 80% aq. ethanol extract was observed 10.65±0.30 mg/gm. The study also revealed that the ABTS radical scavenging activity showed higher antioxidant capabilities with IC_{50} value 0.27±0.003 mg dry extract than DPPH assay (IC_{50} 0.76±0.03 mg dry extract).

3.5. Identification and quantification of phenolic acids and flavonoids in the wild edible fruit by HPLC

A typical HPLC chromatogram of the mixture of all standard phenolic acids and flavonoids recorded at 280 nm is presented in fig. 4.

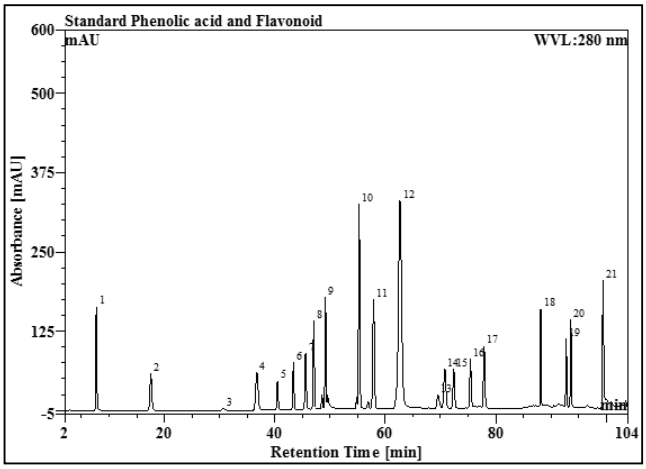

Fig 4: HPLC Chromatogram of mixture of Standard phenolic acids and flavonoids

1. Gallic acid 2. Protocatechuic acid 3. Gentisic acid 4.p-Hydroxy benzoic acid 5.Catechin 6. Chlorogenic acid 7.Vanillic acid 8. Caffeic acid 9. Syringic acid 10. p-Coumaric acid 11. Ferullic acid 12. Sinapic acid 13. Salicylic acid14. Naringin15. Rutin16. Ellagic acid 17. Myricetin 18.Quercetin.19. Naringenin 20. Apigenin 21. Kaempferol

The HPLC method was successfully employed for the identification and quantification of phenolic acids and flavonoids e.g gallic acid, protocatechuic acid, gentisic acid, chlorogenic acid, *p*-hydroxy benzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, sinapic acid, salicylic acid and ellagic acid, catechin, rutin, myricetin, quercetin, naringin, apigenin and kaempferol in the 80 % aq. ethanol extract of *C. hardwickii*. The quantity of all phenolic acids and flavonoids in this fruit has been expressed as mg/100gm dry plant material and data presented in table 3.

Table 3: Phenolic acid and flavonoid content by HPLC mg/100gm dry plant material

Phenolic acids/flavonoids	Amount mg/100gm dry plant material	Phenolic acids/flavonoids	Amount mg/100gm dry plant material	Phenolic acids/flavonoids	Amount mg/100gm dry plant material
Gallic acid	1.03±0.05	Caffeic acid	0.14±0.001	Rutin	0.51±0.04
Protocatechuic acid	0.32±0.003	Syringic acid	1.05±0.07	Ellagic acid	1.09±0.07
Gentisic acid	ND	p-Coumaric acid	0.04±0.0001	Myricetin	ND
p-Hydroxy benzoic acid	0.42±0.001	Ferullic acid	0.06±0.0002	Quercetin	ND
Catechin	ND	Sinapic acid	0.07±0.003	Naringenin	ND
Chlorogenic acid	0.78±0.005	Salicylic acid	ND	Apigenin	ND
Vanillic acid	0.93±0.002	Naringin	0.10±0.002	Kaempferol	1.93±0.06

Each value in the table was obtained by calculating the average of three experiments and data are presented as Mean ± SEM

The HPLC chromatogram of the fruits *C. hardwickii* (Fig.5.) showed the presence of gallic acid

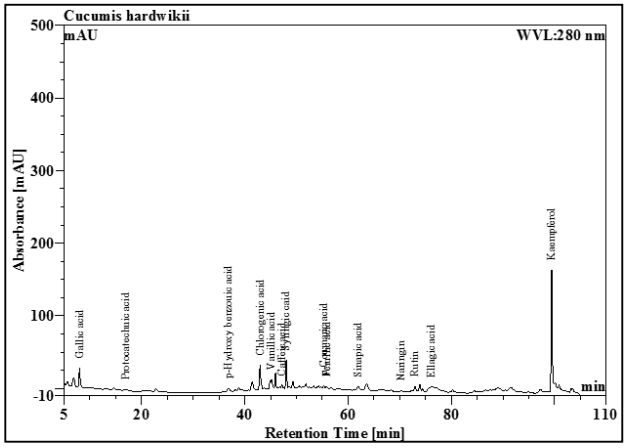

Fig 5: HPLC chromatogram of fruits of *C. hardwickii* showing phenolic acids and flavonoids

(1.03±0.05 mg/100gm), protocatechuic acid (0.32±0.003 mg/100 gm), *p*-hydroxy benzoic acid (0.42±0.001 mg/100gm), chlorogenic acid (0.78±0.005 mg/100gm), vanillic acid (0.93±0.002 mg/100 gm), caffeic acid (0.14±0.001 mg/100 gm), syringic acid (1.05±0.07 mg/100 gm), p-coumaric acid (0.04±0.0001 mg/100 gm), ferulic acid (0.06±0.0002 mg/100 gm), sinapic acid (0.07±0.003 mg/100 gm), naringin (0.10±0.002 mg/100 gm), rutin (0.51±0.04 mg/100 gm), ellagic acid (1.09±0.07 mg/100 gm) and kaempferol (1.93±0.06 mg/100gm).

4. Discussion

The moisture content in a food involves the amount of water present in the food and the moisture content determines the actual quality of the food before consumption. Moisture content affects the physical, chemical aspects of food which relates with the freshness and stability for the storage of the food for a long period of time [22].

The determination of moisture content is also necessary to calculate the content of other food constituents on a uniform basis. The moisture content in *C. hardwickii* was found to be 92.58± 2.35 % and this result was very close to the moisture content of some common vegetables like bitter gourd (92.4%), cucumber (96.3%), broad beans (85.4) etc, grown in India [23].

Estimation of ash content in food is very much important for several reasons. It is part of proximate analysis for nutritional evaluation of food. Ash content represents the total mineral content in foods. Although minerals represent a small proportion of dry matter, often less than 7% of the total, they play an important role from a physicochemical, technological and nutritional point of view [24]. The wild fruits *C. hardwickii* contained high amounts of ash (16.87 ± 1.35%) indicating that this fruits were rich in minerals and could provide a considerable amount of mineral elements in our diet. Fat is an important component of diet and serves a number of functions in the body. Fats provide essential fatty acids, which are not made by the body and must be obtained from food. The essential fatty acids are linoleic and linolenic acid. They are important for controlling inflammation, blood clotting, and brain development. Fat serves as the storage substance for the body's extra calories. Apart from these, the presence of fat in the diet is important for the absorption of fat soluble vitamins like vitamin A and carotene in the body [23]. The fat content in the fruits of *C. hardwickii* (9.4±0.36 %) was particularly high and well compared to that reported for some common vegetables like spinach (0.7 %), lettuce (0.20 %) [25].

Vegetables are rich sources of fiber which plays an important role in decreasing the risks of many disorders such as overweight, constipation, diabetes, serum cholesterol, heart diseases, breast and colon cancer, hypertension, etc [26]. The World Health Organization (WHO) has recommended an intake of 22-23 kg of fibre for every 1000 k. cal. of diet which is necessary for digestion and effective elimination of wastes [27]. The fruits of *C. hardwickii* contained a good amount of crude fibre (20.53 ± 1.55%) and similar to commercial fruits and vegetables like apple (3.2 %), broad beans (8.9 %), cabbage (2.8 %), potato (1.7 %), spinach (2.5 %) [16]. So, these wild vegetables used could be used in the human diet to fulfil WHO recommendation.

Carbohydrates are a class of energy yielding substances which include starch, glucose, cane sugar, milk sugar etc. and it is the major nutrients of fruits and vegetables. The carbohydrate contents of the fruits of *C. hardwickii* has been presented in

Table 1. The carbohydrate content in this fruits (29.67±1.65 %) considerably higher than the reported values when compared to some wild edible vegetables like bitter gourd (10.6 %), beans (29.1 %), potato (20.9 %), jack fruit seeds (25.8%) etc [23]. So this fruit under study could be a good source of carbohydrate for human consumption.

Proteins are one of the most important nutrients required by the body and should be supplied in adequate amounts in the diet. The dietary proteins are broken down into amino acids which are used by the body to synthesise the protein needed by the body for various functions like, for the proper functioning of antibodies resisting infection, for the regulation of enzymes and hormones, for growth, and for the repair of body tissue etc [23]. Legumes, which include beans, lentils, and dried peas, and soy, nuts and seeds, are rich sources of protein. The crude protein content in *C. hardwickii* (11.9± 1.36%) was very close to some wild leafy vegetables such as *Momordica balsamina* (11.29±0.07%), *Carpesium cernuum* (17.22±0.03%), *Eurya acuminata* (14.72±0.04 %) and *Ardisia humilis* (12.71±0.33%) [28-30]. In general, it is also recommended that plant food providing more than 12% of its caloric value from protein is a good source of protein. So, the data (Table1) showed that the vegetables are rich sources of protein which can encourage their use in human diets [24]. The energy value of foods is often more easily calculated from the analysis of foods for proteins, fat and carbohydrates and multiplication of the content of these components with appropriate factors. The results obtained from systematic chemical analysis of *C. hardwickii* established that caloriefic value of this fruits (250.88 ± 2.65 kcal/100gm) were higher than potato (97 kcal/100gm), beans (158 kcal/100gm), jack fruit (133 kcal/100gm) etc [23].

Minerals play a key role in maintaining proper function and good health in the human body. Fruits, and vegetables, are important sources of essential elements. Sodium, potassium, calcium, and magnesium are macro-minerals, whereas iron, zinc, copper, manganese, zinc, lead and chromium are micro-minerals which are also known as trace elements. The trace elements are required in very trace quantities as they are important for the physiological and biological functions of the human body. The essential and trace minerals of edible parts of *C. hardwickii* are shown in Table 1. The fruits contain a very good amount of sodium and potassium. Na and K take part in ionic balance of the human body and maintain tissue excitability. Na plays an important role in the transport of metabolites and K is important for its diuretic nature. The K/Na ratio in our body is of great concern to prevent high blood pressure and the ratio should be greater than one because K depresses and Na enhances blood pressure [31]. The ratio of K/Na was significant in this fruit (66.5 and very much compared with some common fruits (Amla 45, papaya ripe 11.5, tomato 11.31 and *Castanea sativa* 56.67 [25] and so the consumption of these vegetables are helpful for human and might be able to control the high blood pressure of our body.

The Ca is an important macro-nutrient which constitutes a large proportion to the bone, human blood and extracellular fluid. It is also very much required for the normal functioning of the cardiac muscles, blood coagulation, milk clotting and the regulation of cell permeability [12]. The fruits of C. hardwickii found to contain 28.0 ±1.54 mg/g calcium which is very much comparable with some cultivated vegetables, and fruits, indicating that the wild vegetables used in this study could provide a good source of Ca to our diet.

Copper (Cu) is an essential trace element that cannot be formed by the human body. It exists as an important component of an enzyme that helps the incorporation of iron into red blood cells, preventing anaemia whereas Zn play a central role in nucleic acid metabolism and act as a membrane stabilizer the deficiency of which leads to growth failure and poor development of gonadal function [32]. A sufficient amount of Cu and Zn were present in the fruits of *C. hardwickii* (0.0017 ±0.0002 and 0.026 ±0.0003 mg/g respectively). The Cu levels in the wild vegetables presented in our study were very much comparable with some common vegetables like broad beans (0.0017mg/g), brinzal (0.0012 mg/g), cucumber (0.0009 mg/g) etc [23].

Fe is important in the diet for the formation of hemoglobin, and normal functioning of the central nervous system. It is a component of muscle and blood and is essential to carry oxygen around the body. Apart from that both Fe and Mn plays an important role in the metabolism of protein, carbohydrate and fats. Regular consumption of iron rich vegetables can prevent the iron- deficiency anaemia [26]. An appreciable amount of Fe (0.229 ±0.25 mg/gm) and Mn (0.0127 ±0.0005 mg/gm) were detected in the fruits of *C. hardwickii*.

A very good quantity of Mg was also present in *C. hardwickii* (0.55 ± 0.12 mg/g). Magnesium is very much essential in a human body to maintain normal nerve and muscle function. So the regular consumption of this magnesium rich vegetables, control the blood- glucose levels and support a healthy immune system [33].

The heavy metals content of the wild vegetables under investigation has listed in Table 1. Among heavy metals lead (Pb) is a potential pollutant that readily accumulates in soils and sediments. Although Pb is not an essential element for plants it gets easily absorbed and accumulated in different plants parts. The consumption of vegetables containing Pb causes both acute and chronic poisoning. It has adverse effect on liver, kidney, vascular and immune system [34] whereas chromium is an essential trace element that enhances insulin function and influences carbohydrate, protein and fat metabolism but chronic exposure to Cr may damage liver and kidney. The concentration of Pb was found in this plant (0.00017±0.001 mg/g) which is lower than the WHO permissible limit of 0.0003 mg/g. The level of Pb reported in this study is comparable to those reported for Indian basil (0.00009 mg/g), bitter leaf (0.00014 mg/g), cabbage (0.00013 mg/g) and water leaf (0.00018 mg/g).

In present study the Cr content (0.0012 ± 0.024 mg/gm) in the vegetables were found lower when compared to the WHO permissible limit of 0.0023 mg/g. The concentration of Cr detected in this study is comparable to those reported for some leafy vegetables such as *Lasia spinosa* (0.0017± 0.001mg/g), *Polygonum microcephalum* (0.00054 ± 0.010 mg/g), *Colocasia esculenta* (0.00062 ± 0.002 mg/g), *Centella asiatica* (0.00097 ± 0.007mg/g) etc [33].

In the present investigation, the water soluble B vitamins and vitamin C content were analysed using HPLC. Vitamin C is the most important vitamin in fruits and vegetables. It is well-known for its antioxidant properties and it helps the body in inhibiting from viral infection, bacterial infections and toxicity. It is required for the prevention of scurvy and maintenance of healthy skin, gums and blood vessels and the deficiency of this vitamin causes bruising, bleeding, dry skin and depression [35].

Due to having high amount of vitamin C this plant might be beneficial to reduce the risk of atherosclerosis and some forms of cancer [36].

Thiamine (B1), is an essential nutrient required by the body

for maintaining cellular function and consequently a wide array of organ functions whereas riboflavin (B2) is the counterpart to thiamine used in the strengthening of food products [37].

Vitamin B3 and B9 are water-soluble B vitamin with many rich natural sources. Both these vitamins are required for numerous body functions including DNA synthesis and repair, cell division, and cell growth whereas vitamin B5 is an essential vitamin required by the body for cellular processes and optimal maintenance of fat. The deficiency of these vitamins can lead to anemia in adults, and slower development in children and also responsible for irritability, fatigue, apathy, numbness, paresthesia, and muscle cramps in human being [38-42].

Pyridoxine (B6) is another water soluble vitamin necessary for the proper maintenance of red blood cell metabolism, the nervous system, the immune system, and many other bodily functions [43].

The amount of vitamin C, B1, B2, B3, B5, B6 and B9 detected in the fruits of *C. hardwickii* were sufficiently high and comparable with some common fruits and vegetables. Therefore, the regular consumption of this vegetable would supply adequate vitamins necessary to maintain healthy body functions and also plays an important role as an antioxidant *in vivo*, both by preventing the adverse effect of reactive oxygen species (ROS), as well as by inhibiting lipid peroxidation [44].

In this study the 80% aq. ethanol extract of *C. hardwickii* showed potent antioxidant activities using DPPH and ABTS assay. The IC50 value of DPPH assay was found to be higher than that of ABTS assay which showed more antioxidant activities. The total phenolic content obtained in the plant is more than the flavonoid and flavonol content. The concentration of total phenolic and flavonoid in plant extracts depends on the polarity of solvents used in the extract preparation. It has been established that phenolic compounds are the major plant compounds with antioxidant activity and this activity is due to their redox properties. Phenolic compounds are a class of antioxidant agents which can adsorb and neutralize the free radicals [45]. Flavonoids and flavonols are regarded as one of the most widespread groups of natural constituents found in the plants. It has been recognized that both flavonoids and flavonols show antioxidant activity through scavenging or chelating process [46]. The results strongly suggest that phenolics are important components of these plants. The other phenolic compounds such as flavonoids, flavonols, which contain hydroxyls are responsible for the radical scavenging effect in the plants.

The antioxidant activities of the extractive solution represent an important parameter to evaluate the biological property of the plant. Therefore, it is necessary to characterize and quantify the important compounds like phenolic acids and flavonoids present in the plant and also to validate the method of separation and identification of active constituents.

The HPLC analysis showed the presence of gallic acid which remains in the plant either in the Free State or in the combined form as ester and acts as a powerful antioxidant. Protocatechuic acid is a type of widely distributed naturally occurring phenolic acid. It has structural similarity with gallic acid, caffeic acid, vanillic acid and syringic acid which are well-known antioxidant compounds. A good amount of these phenolics detected in this plant might be responsible for the strong antioxidant properties of the plant and thus help in prevention and therapy of various oxidative stress related diseases such as neurodegenerative and hepatic diseases [47].

p-Hydroxybenzoic acid, reported to possesses antifungal, anti-mutagenic, anti-sickling, estrogenic, and anti-microbial activities whereas chlorogenic acids has been established to reduce blood sugar levels and potentially exert an anti-diabetic effect. The consumption of the plant containing these phenolic acids is associated with a lower risk of a variety of liver diseases, including liver cirrhosis and liver cancer [48].

p-coumaric acid, well-documented for its antioxidant behaviour, is widely distributed in food stuffs, such as, barley, peanuts, navy beans, tomato, carrots etc. and is believed to have antioxidant behavior thereby reducing the formation of carcinogenic nitrosamines in the stomach [49].

One of the important phenolics, ferulic acid which is detected in the 80 % aq. ethanol extract of *C. hardwickii* in our study, is well-known for its physiology functions, such as, anti-microbial, anti-microbial, anti-inflammatory, anti-cancer activities etc. It also lowers cholesterol level in serum and increases sperm viability [50].

Sinapic acid is a substance widely prevalent in the plant kingdom and it showed antioxidant, anti-microbial, anti-inflammatory, anticancer, and anti- anxiety activity whereas Ellagic acid is a natural phenol antioxidant found in numerous fruits and vegetables and have a variety of benefits like anti-mutagenic, antimicrobial and antioxidant properties, and inhibitors of human immunodeficiency virus (HIV) [51]. A very significant amount of sinapic and ellagic acid were detected in the plant under investigation and consumption of this plant would be useful for health promotion.

Naringin is a flavanone 7-O-glycoside found in grapes and citrus fruits. Several investigations also suggest that naringin supplementations are beneficial for the treatment of obesity, diabetes, hypertension, and metabolic syndrome [52]. The HPLC analysis of the ethanol extract of *C. hardwickii* showed the presence of good amount of naringin (0.10±0.002 mg/100gm), rutin (0.51±0.04 mg/100gm) and significant amount of kaempferol (1.93±0.06 mg/100gm). Rutin is a phenolic compound with glycosidic linkage. It is reported to exhibit significant pharmacological activities, including anti-oxidation, anti-inflammation, anti-diabetic etc whereas kaempferol, is a flavonol found in many edible plants and is reported to possess potent pharmacological and nutraceutical activities. The rutin content in the plant extract were comparable to that in the leaves of *Fagopyrum esculentum* (0.12 mg/gm) and *Melisa officinalis* (0.30 mg/gm). The consumption of plants containing rutin kaempferol thereby conferring innumerable health benefits in the form of reducing scourge of cardio vascular diseases, cancer, arteriosclerosis etc [53-54].

5. Conclusion

The investigation showed that this wild edible plant collected from Uttarakhand state in India are rich in protein, fat, carbohydrate, fibre, and vitamins and could provide essential nutrients required for maintaining normal body function. The nutritional property of this plant was similar to and also sometimes better than the common vegetables. The fruit was also found to be a significantly useful source of various minerals. The minerals, particularly Na, K, Ca, Fe, Cu, Mg and Zn, were present in appreciable quantities. The toxic heavy metals Cd and Hg were not detected in the plant materials but Pb and Cr were detected within the permissible limit as prescribed by WHO and the presence of the heavy metals in the plant might not be harmful for mankind. The antioxidant properties and the presence of various phenolic acids and flavonoids inferred that the fruits of this plant could be used for the nutritional purpose of human being and

adequate protection may be obtained against diseases arising from malnutrition.

The reversed-phase HPLC method with diode array detection was developed for the quantitative estimation of vitamins, phenolic acids and flavonoids. The established HPLC assay showed a well separation of the compounds and also the developed method was linear, sensitive, accurate, meticulous and reproducible. Therefore, the method is suitable for the simultaneous determination of phenolic acids and flavonoids in different formulations with 'shorter run time' and 'high efficiency'. The presence of significant amount of respective bio-active components in this plant under study and variation of quantity determined ensures its unequivocal recommendation for the use in the pharmaceutical and nutraceutical sector.

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