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Research article

Celosia cristata Linn. flowers as a new source of nutraceuticals- A study on nutritional composition, chemical characterization and *in-vitro* antioxidant capacity

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ABSTRACT

This study was undertaken to investigate the nutritional value, chemical characterization and *in-vitro* antioxidant activity of *Celosia cristata* Linn. inflorescences, a culturally significant plant of Kashmir valley, India. The results revealed that the flower contained variety of vitamins (A, B-complex, C and E) with Vitamin E (tocopherol) showing the highest concentration. Among minerals, potassium was found to be present in significant amounts, the amino acid and fatty acid profile of the flower was also found to be satisfactory. The antioxidant activity of flower extract was evaluated by various *in-vitro* analytical methods: DPPH free radical scavenging activity, lipid peroxidation, reducing power, and metal chelating ability. Therefore, the present research brings into focus, the nutritional and antioxidant potential of *C. cristata* flower and its extract.

1. Introduction

Medicinal plants are indispensable sources of bioactive compounds and have proved to be stalwart ingredients for a wide range of applications such as food additives, cosmetics, pharmaceuticals, agrochemicals, repellants, ethno-medicines etc (Sayeed and Thakur, 2019). Over the last few decades, many medicinal plants, herbs and flowers have been investigated for the presence of various phytochemicals having antioxidant activities (Sayeed and Thakur, 2019). These include ascorbic acid, tocopherol, phenolic compounds etc. These naturally-derived antioxidants are gaining increasing attention of the researchers and food manufacturers because of their abilities to act as reducing agents, chelators of pro-oxidant metals and to quench free radicals. Increased consumption of antioxidants and phenolic compounds has been associated with reduced risk of degenerative "oxidative" diseases. The valley of Kashmir that lies in the northern part of India has a rich diversity of medicinal flowers, plants, and herbs. There is a documented evidence of 1,748 medicinal plant species from the region (Dar et al., 1984). While the majority of these plants could be found in the wild, others are cultivable. The plant, *Celosia cristata*, commonly known as cock's comb flower, is one of the valued medicinal plants of the Kashmir valley (Sayeed and Thakur, 2019).

Locally known as "Mawal", *C. cristata* is an herbaceous plant belonging to the family Amaranthaceae (Caryophyllales). It is grown as an ornamental plant in most of the parts of the world because of the attractive and vibrant colored inflorescences. In some parts of the World like Africa, China, Indonesia, India, and other parts of Asia, its leaves and inflorescences are eaten as vegetables (National Research Council, 2006). The plant, in general, was found to contain phenolic compounds, tannins, flavonoids, and sterols. Various authors have published reports on identification and isolation of different compounds from the seeds and leaves of the plant. These include saponins (cristatain, celosin A, celosin B, celosin C, and celosin D, semenoside A), glycoproteins (CCP-25 and CCP-27), 4-hydroxyphenethyl alcohol, β-sitosterol, 2-hydrox-yoctadecanoic acid, and stigmasterol (Gholizadeh and Kapoor, 2004; Chi et al., 2010; Wang et al., 2010). The dried inflorescences, seeds, and leaves are reportedly used in African, Indian (Ayurvedic and Yunani) and Chinese traditional medicinal systems to cure various ailments (Kirtikar and Basu, 1918; National Research Council, 2006). For use in ethnomedicine different parts of *C. cristata* are used by traditional healers. The dried red flowers are effective against hematuria, abdominal pains, leucorrhea, heavy menstrual bleeding, hemoptysis, hemorrhoid bleeding and osteoporosis (Anderson, 1986; Foster and Chongxi, 1992; Motaleb et al., 2015). Apart from its use in folklore medicine, scientific investigations have reported its hepatoprotective, antimicrobial, anthelmintic,

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antimelanocyte, antinoceceptive, anti-oxidant, anti-aging and adipogenesis reduction activities (Yun et al., 2008; Wang et al., 2010; Woo et al., 2011; Jayanthy and Shafna, 2012; Fitoussi et al., 2013; Islam et al., 2016). Traditional healers (hakims) practicing in the Kashmir Valley mostly follow the Yunani system of medicine and use dried, powdered red inflorescences and seeds of *C. cristata* in accurately measured proportions (depending upon the ailment being targeted) in most of their traditional remedies. Besides being used in traditional medicine, the inhabitants of the Kashmir valley have found a novel way of using this edible flower. It is used as a natural food colorant in local dishes to impart a fiery hot color to gravies, without affecting taste and aroma. The coloring property of *C. cristata* flower is due to the presence of betalains, which are water soluble pigments found in plants belonging to the family Amaranthaceae (Cai et al., 1998). Although the cultural significance of the flower is high, to our best knowledge no study has been carried out to assess the nutritional composition, phytochemicals and antioxidant activity of red inflorescences of *C. cristata* found in Kashmir. In the present study, our aim was to investigate the nutrient, bioactive composition and antioxidant activity of this underexploited flower grown in Kashmir valley.

2. Material and methods

2.1. Collection of raw material

The indigenous variety of *Celosia cristata* Linn. was procured from the herbarium of SKUAST-K (Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir), Kashmir, India with Herbarium No: CCI7001. The flowers were separated from the stalks, leaves were removed and then threshed to remove the seeds and the adherent dirt. The cleaned flowers excluding seeds were hung upside down in bunches and shade dried under the roof to retain the bioactive components. The flowers were harvested in the month of October 2018.

2.2. Reagents used

All reagents and chemicals used were of analytical grade. DPPH, ferrozine, analytical standards of FAMEs, pyrogallol and linoliec acid were procured from Sigma– Aldrich Co. (St. Louis, MO, USA). Sodium acetate, HPLC-grade methanol, acetonitrile, tetra hydrofuran (THF) and formic acid were purchased from Merck (Mumbai, India).

2.3. Proximate analysis

Fresh *C. cristata* flowers (CCF) were used for carrying out the proximate composition using standard protocols of AOAC (2006). Crude protein was calculated using nitrogen to protein conversion factor of 6.25 (Kjeldahl Method). Total carbohydrate content was calculated by difference using the formula, 100 – (Protein + Fat + Ash + Moisture). Total energy was calculated by using Atwater according to the given equation: Energy (kcal) = 4 (g protein + g carbohydrate) + 9 (g lipid). Each analysis was conducted in triplicates.

2.4. CCF extract preparation

The CCF was first dried using traditional drying technique. The cleaned flowers excluding seeds were hung upside down in bunch and shade dried under the roof so as to retain the biologically active ingredients. The dried CCF was subjected to pulverization to obtain finely ground powder. This powdered CCF was extracted with 70% ethanol (v/v) at room temperature (27 °C) in dark for 24 h. The extraction was carried twice and the combined extracts were filtered through Whatman No. 1 and centrifuged at 10000 rpm for 15 min. The supernatant was concentrated under vacuum in rotary vacuum evaporator (HAHNSHIN, South Korea) at 35 °C. The resulting extract was stored at 4 °C until use.

2.5. Vitamin and mineral analysis of CCF

Vitamin C content of CCF was determined using 2,6-dichloroindophenol titrimetric method, AOAC Method 967.21 (2007). For determining the content of Vitamin B- complex, the sample preparation was done by adopting Ultrasonic assisted extraction method (UAE) which enhanced the extraction efficiency of B-complex vitamins. The prepared sample was analyzed for quantification of B- complex vitamins according to AACC Method 86–90.01 (1995), using UPLC coupled with Xevo TQD MS (Acquity, H- Class System, Waters Corporation, Massachusetts, United States). An Acquity UPLC BEH C-18 column (2.1 mm × 50 mm i. d., 1.7μm) was used for chromatographic separation of B-complex vitamins. The Binary Eluent composed of acetonitrile and water containing 0.1% formic acid. For Vitamin A (retinol) and E (a-tocopherol) analysis, the methodology illustrated by Rizzolo and Polesello (1992) was adopted. The sample was saponified with 50% ethanolic KOH with the addition of pyragallol as antioxidant and refluxed for 1 h. After cooling tetrahydrofuran (THF) mixed with water (1:1) was added and the mixture was kept for 24hours at room temperature (27 °C). Separation and quantification were achieved by reversed phase HPLC- DAD (Agilent 1260 Infinity Diode Array Detector, Agilent technologies, Santa Clara, California, USA) loaded with C-18 column (4.6 mm × 250 mm i. d., 5μm). Methanol:-Water (96:4) was used as a mobile phase. 20μl injection volume was used for determination and 1.5ml was transferred into the autosampler. The run length of the method was 14 min.

For determining the content of mineral ion the sample was prepared using wet digestion method described by Pequerul et al. (1993) with different concentration. The digestion was done with HNO_3 (supra-pure 60–70%). The resulting sample was analyzed for various metals (Ca, Zn, Fe, K, Na, Mg and Cu) in an inductively coupled plasma-optical emission spectrophotometer (ICP-OES, Optima 8000, Perkin Elmer, USA).

2.6. Fatty acid profiling of CCF

The standard AOAC Method 996:06 (2001) was adopted for extraction of sample for fatty acids analysis, followed by their methylation to fatty acid methyl esters (FAMEs) using methanolic boron trifluoride (BF_3) as an acid catalyst. FAMEs were measured quantitatively by a Gas Chromatograph, GC-FID (Agilent 6890 N, Agilent technologies, Santa Clara, California, USA). A DB-23 column (30 m × 0.25 mm i. d., 0.25μm, Agilent Technologies, Santa Clara, USA) containing 23% methyl-polysiloxane was used as stationary phase for fatty acid analysis. Nitrogen was used as a carrier gas.

2.7. Amino acid profiling of CCF

A reversed phase high performance liquid chromatographic method was adopted to analyze amino-acid content of fresh CCF. The sample was derivatized for volatility suitable for amino-acid analyzing using FMOC-Cl (9- fluorenylmethylchloroformate) as derivatization reagent and analyzed using Agilent 1260 Infinity HPLC System (Agilent Technologies, Santa Clara, California, USA) (Fabiani et al., 2002; Thakur and Sayeed, 2014). Detection was carried out using a Diode Array Detector (DAD, λmax = 263nm). The sample was injected onto a C-18 column (4.6mm × 250 mmi.d., 5μm) equipped with a guard column of the same material (Merck). The column operated at a flow rate of 1 ml/min using acetonitrile and water (90:10) as eluent A and sodium acetate (50mM) as eluent B.

2.8. Preliminary qualitative phytochemical screening

The ethanolic extract of CCF was examined for qualitative screening of various phytochemicals as per standard procedures (Sofowora, 1996; Trease and Evans, 2002; Thakur and Shah, 2016). The preliminary tests in the extracts were conducted to confirm the presence of anthocyanins,

anthraquinones, alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins and amino acids, saponins, steroids, and terpenoids.

2.9. Total phenolic content of CCF

The total phenolic content in methanolic extracts of grounded sample was estimated by Folin-Ciocalteu reagent Stintzing et al., 2005; Thakur et al., 2014).). One hundred mg of gallic acid was dissolved in 100 ml ethanol to prepare Gallic acid stock solution (1000 μg/ml). Various dilutions of standard gallic acid were prepared from this stock solution. One millilitre aliquots of 1.0, 2.5, 5.0, 10, 25, 50 and 100 μg/ml of gallic acid solution were mixed with 5.0 ml of Folin-Ciocalteu reagent (diluted ten-fold) and 4.0 ml of sodium carbonate solution (75 g/l) and calibration curve was plotted. The absorbance was measured after 30 min at 20 °C at 765 nm. One millilitre extract was mixed separately with the same reagents and absorbance was measured at 765 nm after 1 h and kept under light protection. The total phenolic compound in the extract was determined using the formula:

$$C = C_1 \times V/_m$$

C = Total content of phenolic compounds in mg/g in GAE (Gallic acid equivalent), it means the total reduced capacity; C_1 = The concentration of gallic acid established from the standard curve in mg/ml; V=The volume of extract in ml, M = Weight of extract in grams.

2.10. Antioxidant activity

2.10.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of CCF

The ability of CCF extract to scavenge the DPPH radicals was investigated by using standard methodology (Blois, 1958; Shah and Thakur, 2015). Briefly, 0.1 mM solution of DPPH in ethanol and 1.5 ml of this solution was added to 0.5 ml of extract solution in ethanol at different concentrations (50–300 μl/ml). The mixture was shaken and allowed to stand at room temperature (27 °C) for 30 min. The absorbance was then measured at 517 nm using a spectrophotometer. A blank without DPPH was used to remove the influence of the color of the extracts and an ethanolic solution of DPPH was used as a negative control. Ascorbic acid was used as a reference. All the measures were carried out in triplicates. The DPPH radical scavenging activity was calculated using the following equation:

$$\%\ \text{Scavenging effect} = A_0 - \frac{A_s}{A_0} \times 100$$

where,

A_o = Absorbance of negative control
A_s = Absorbance of sample

2.10.2. Ferrous ions and (Fe^{2+}) chelating activity of CCF

Metal chelating ability of CCF extract at different concentrations was determined according to the procedure reported by Dinis et al. (1994).

2.10.3. Reducing power activity of CCF

The reducing ability of CCF extract was determined by adopting method described by Oyetayo et al. (2009).

2.10.4. Inhibition of lipid peroxidation by CCF extract

The antioxidant power of CCF extract at different concentrations was determined by thiocyanate method (Osawa and Namiki, 1981).

2.11. Statistical analysis

Data were reported as mean ± standard deviation. To determine difference among the mean values at 5% significance level, one-way analysis of variance (ANOVA) were computed using a commercial statistical package SPSS 10.1 (USA) by Duncan's multiple range tests.

Table 1. Proximate composition, vitamin content, mineral content, fatty acid and amino aci composition of Kashmiri *Celosia cristata* flowers (CCF).

Analysis	CCF
Proximate composition (g/100 g of fresh weight)	
Moisture	79.62 ± 1.13
Crude Fat	4.58 ± 0.10
Crude Protein	3.56 ± 0.05
Ash	2.44 ± 0.09
Total carbohydrates	9.80 ± 1.40
Energy (Kcal/100gm)	94.74 ± 0.02
Vitamin Composition	
Ascorbic acid (Vitamin C)	4.3 ± 0.06
Retinol (Vitamin A)	11.94 ± 0.36
Tocopherol (Vitamin E)	455 ± 0.01
Thiamine (Vitamin B1)	5.29 ± 0.08
Riboflavin (Vitamin B2)	27.31 ± 0.40
Nicotinamide (Vitamin B3)	49.98 ± 0.19
Panthothenic Acid (Vitamin B5)	107.64 ± 0.10
Pyridoxin(Vitamin B6)	50.60 ± 0.14
Biotine (Vitamin B7)	1.66 ± 0.02
Folic Acid (Vitamin B9)	3.63 ± 0.17
Cyanocobalamine(Vitamin B12)	1.41 ± 0.31
Mineral Content (mg/100g)	
Calcium	128.68 ± 1.53
Zinc	0.90 ± 0.02
Iron	9.45 ± 0.19
Potassium	520.4 ± 1.12
Sodium	3.30 ± 0.02
Magnesium	76.40 ± 0.52
Copper	0.10 ± 0.98
Fatty Acid Composition (mg/100g)	
Palmitic (C16)	1302.5 ± 0.99
Palmetoleic (C16:1c)	5.5 ± 0.03
Stearic (C18)	179 ± 0.40
Oleic (C18:1c)	897.5 ± 0.11
Linoleic (C18:2c)	2027.9 ± 0.06
Linolenic (C18:3c)	167.5 ± 0.34
Total Saturated fat (g/100g)	**1.481** ± 0.04
Total Monounsaturated fat (g/100g)	**0.903** ± 0.30
Total polyunsaturated fat (g/100g)	**2.195** ± 0.22
Amino Acid Composition (mg/100g)	
Histidine	2.99 ± 1.18
Threonine	19.08 ± 0.97
Valine	14.07 ± 1.20
Methionine	0.79 ± 0.87
Tryptophan	26.29 ± 0.16
Phenylalanine	5.73 ± 0.57
Isoleucine	8.83 ± 0.11
Leucine	11.74 ± 0.97
Lysine	17.81 ± 0.56
L-arginine	22.43 ± 0.15
Alanine	16.41 ± 1.01
Proline	10.86 ± 0.32

Results are the mean value of three independent determinations (each one by triplicate) ± standard deviation. The results of Vitamin analysis are expressed in μg/100g, except for Vitamin C which is expressed in mg/100g.

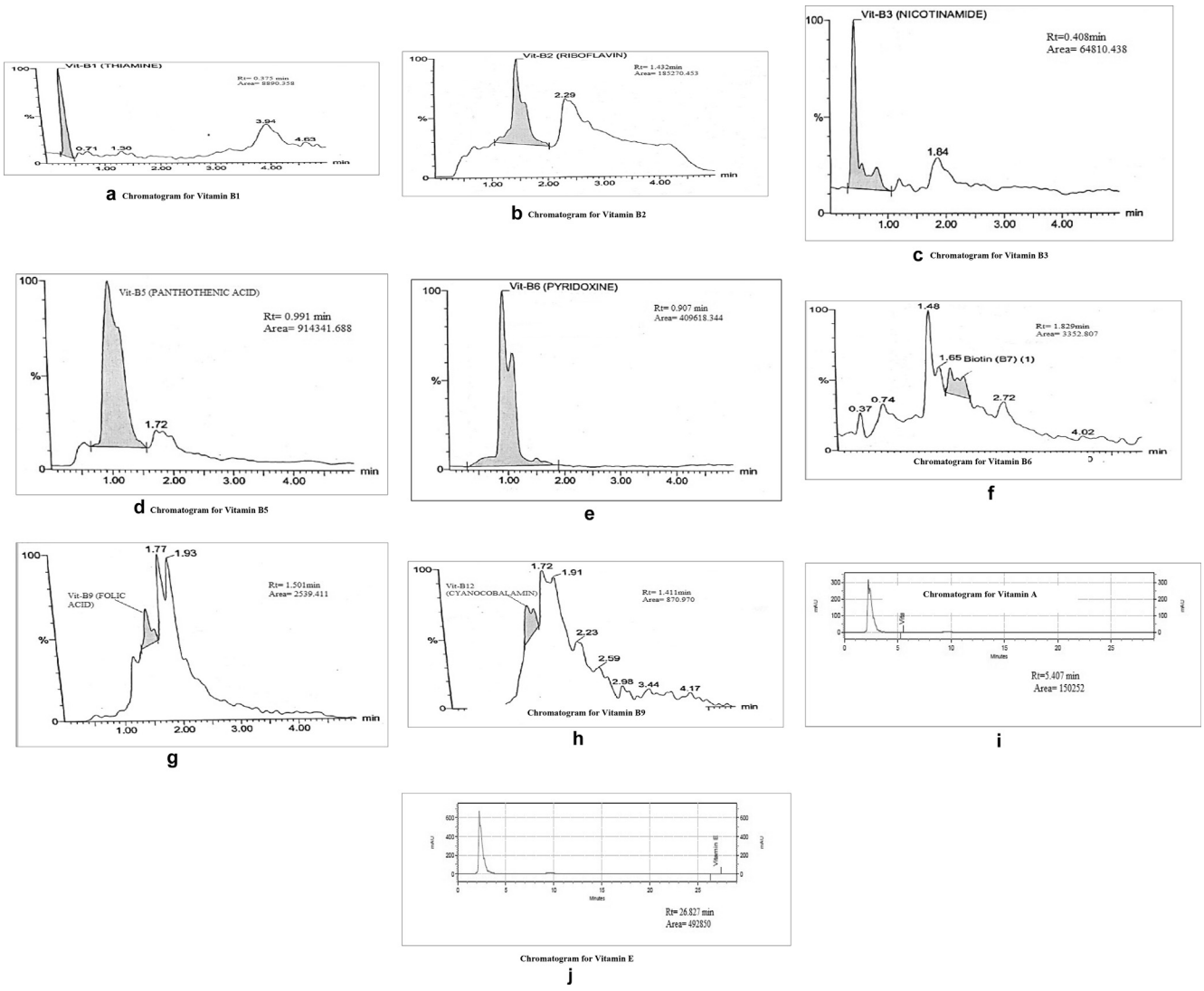

Figure 1. Chromatograms for vitamins; vitamin B1 (a). Vitamin B2 (b). Vitamin B3 (c). Vitamin B5 (d). Vitamin B6 (e). Vitamin B7 (f). Vitamin B9 (g). Vitamin B12 (h). Vitamin A (i). Vitamin E (j).

3. Results and discussion

3.1. Proximate composition and energy value of CCF

The results of the proximate composition and energy value of fresh CCF are presented in Table 1. The edible flowers of *C. cristata* showed high moisture content (79.6 g/100g). Total carbohydrates (9.80g/100g) was the most abundantly found macronutrient in fresh CCF. The results revealed higher mineral concentration in CCF than reported in other edible flowers (Navarro-González et al., 2014; Prata et al., 2017; Rachkeeree et al., 2018).

3.2. Vitamin and mineral profiling of CCF

The composition of various vitamins and mineral elements is shown in Table 1. Vitamins are the essential micronutrients which orchestrate various physiological functions in the human body. These essential components need to be supplied from the diet to prevent deficiencies (hypovitaminosis or avitaminosis) (Maggini et al., 2018). In the present investigation, an attempt was made to quantify the vitamins (A, B-complex, C and E) present in *C. cristata* inflorescences. The chromatograms of vitamins as obtained by HPLC are presented in Figure 1 (a–j). Water soluble vitamin, Vitamin C (ascorbic acid) was found to be present in CCF at a concentration of 4.3 mg/100gm. This value is very low if compared with other edible flowers (Prata et al., 2017). Among B-complex vitamins, Panthothenic acid (Vitamin B5) was found to be present in adequate amounts (107.64μg/100g) followed by Pyridoxine (Vitamin B6). Thiamine (Vitamin B1), Folic acid (Vitamin B9), Cyanobobalamine (Vitamin B12) and Biotine (Vitamin B7) were present in trace amounts. Lipophilic Vitamin, Vitamin A (Retinol) was present in minute amounts (11.95μg/100g). High concentration of Vitamin E (α-tocopherol), which is an important antioxidant, was present in the sample (455.00μg/100g). Compared to other plant resources, the concentration of vitamin E was higher and can play a pivotal role in providing the essential micronutrients to the consumer.

Potassium concentration exceeded all mineral elements at 520.45 mg/100g followed by Calcium at 128.68 mg/100g. Relatively high amounts of Magnesium (76.40 mg/100g) and Iron (9.45 mg/100g) were also present in the flowers. Also, Copper, Zinc and Sodium were present but in lower amounts. Our results reveal higher mineral concentration in CCF than reported in other edible flowers (Navarro-González et al., 2014; Prata et al., 2017; Rachkeeree et al., 2018). This explains the role played by *C. cristata* flowers in ethnomedicine for the treatment of various ailments like osteoporosis, leucorrhea, heart diseases, wound healing etc.

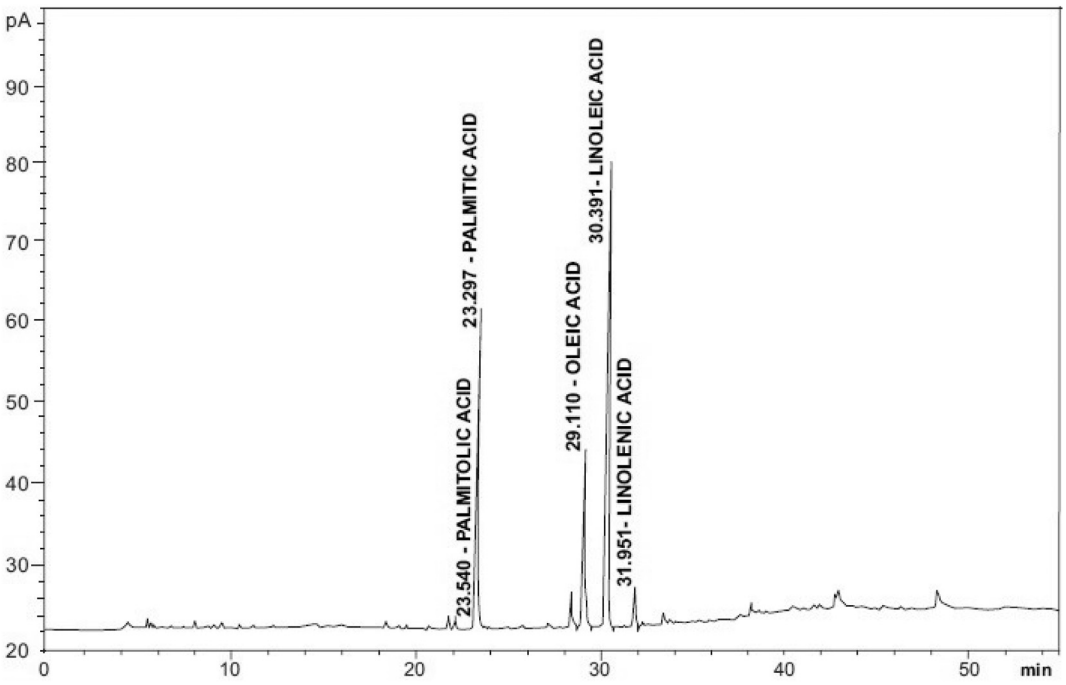

Figure 2. Fatty acid profile of *Celosia cristata* flowers (on fresh weight basis).

3.3. Fatty acid profiling of CCF

The results for fatty acid composition, total saturated fat, monounsaturated fat and polyunsaturated fat of CCF are shown in Table 1 and the chromatograms are presented in Figure 2. Linoleic acid (C18: 2c), a polyunsaturated omega 6- fatty acid, was the most predominant polyunsaturated fatty acid (PUFA) present in the flower sample (2.02g/100g) followed by α-linolenic acid (C18:3c). The fatty acids belonging to n-3 and n-6 series are reported to have beneficial health effects. Among monounsaturated fatty acids (MUFA), oleic acid (C18:1c, 0.89g/100g) was found in moderate amounts. The saturated fatty acid (SFA) fraction consisted mainly of palmitic acid (C16:0, 1.3g/100g). The results of fatty acid profiling of CCF are in accordance with those reported by other authors where, linoleic and palmitic acids predominated the PUFA and SFA fractions in edible flower sample (Pires et al., 2017).

3.4. Amino acid profiling of CCF

The *C. cristata* inflorescences were analyzed for the composition of various "essential" and non-"essential" amino-acids. The results of the study are summarized in Table 1. As can be depicted clearly from Figure 3, twelve amino- acids were detected and quantified which included 10 essential and two non-essential (alanine and proline) amino acids. Among essential amino- acids, tryptophan (precursor of serotonin synthesis) was found in appreciable amounts (26.29mg/100g) in the sample followed by L- arginine and threonine at a concentration of 22.43 mg/100g and 19.08 mg/100g respectively. Branched chain amino acids (BCAAs) i.e., leucine, isoleucine and valine were found in moderate amounts. These benefit muscle growth by stimulating protein synthesis and decreasing muscle fatigue. Methionine exhibited the lowest concentration of 0.79 mg/100g. Alanine and proline were the two non-essential amino acids found in the sample. All of these amino acids are required by the body to regulate hormonal secretion and act as ergogenic aids.

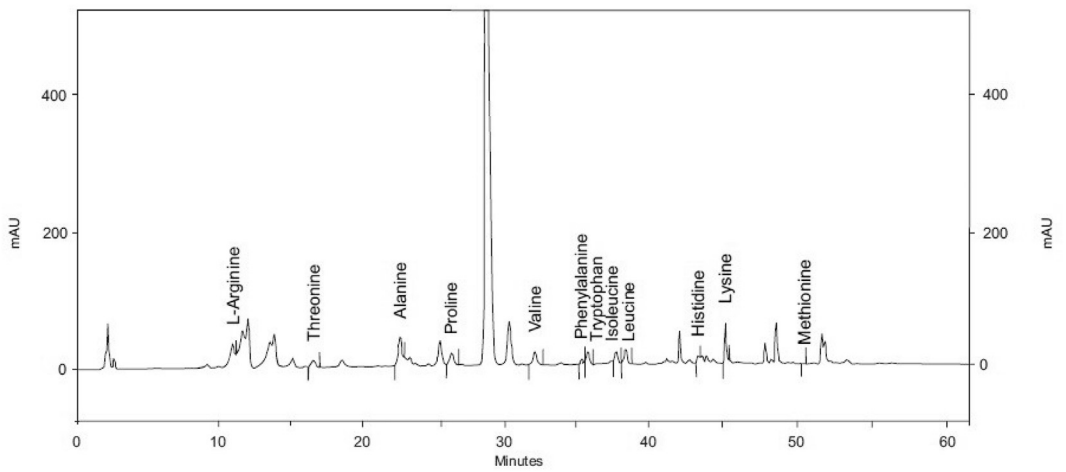

Figure 3. Amino acid profile of *Celosia cristata* flowers (on fresh weight basis).

Table 2. Qualitative phytochemical screening of CCF.

Phytochemicals	Tests	CCF
Alkaloids	Mayer's Test	+
Anthraquinones	Borntrager's Test	-
Anthocyanins	Sodium Hydroxide Test	-
Carbohydrates	Molisch's Test	+
Flavonoids	Shinoda Test	+ +
Glycosides	Keller-Kiliani's Test	+
Phenols	Ferric chloride Test	+
Proteins and amino acids	Xanthoproteic Test	+
Saponins	Foam Test	+ +
Steroids	Liebermann-Burchard's Test	-
Tannins	Ferric Chloride Test	+
Terpenoids	Salkowski test	-

(-): Absence; (+): Moderate presence; (++): Strong presence.

3.5. Qualitative phytochemical screening and total phenolic content of CCF

Our investigation revealed the presence of various primary and secondary metabolites in the CCF and the results are provided in Table 2. These bio-active components are proven anti-oxidant, anti-bacterial and anti-cancerous agents. Steroids, anthocyanins, anthraquinones and terpenoids were not found in the sample. The flower contained higher amounts of flavonoids and saponins. The results are in agreement with those reported previously (Islam et al., 2016; Woo et al., 2011). Earlier studies have reported that alkaloids, tannins, and phenolic compounds might be responsible for anthelmintic properties exhibited by plant extracts (Suman et al., 2011), explaining its use as an anthelmintic. Flavonoids and saponins possess numerous health benefits like ability to scavenge free radicals, blood lipid modulation, reducing inflammation, thus supporting its use as an antioxidant, anti-inflammatory agent and in reducing the risk of CVDs, The amount of total phenols in CCF extract was 47.29 mg GAE/g which was more than that stated in previous reports on the same flower (Woo et al., 2011) as well as other edible flowers (Rachkeeree et al., 2018). Our findings are in agreement with the authors who have reported almost same values for total phenolic content in aqueous and alcoholic extracts of *C. cristata* (Islam et al., 2016; Kim et al., 2012, 2015)

3.6. Antioxidant potential of CCF

For determining the antioxidant activity (AOA) of CCF extracts, various *in-vitro* analytical methods were employed to measure various oxidation products, i.e., measuring the decrease in absorbance of the sample containing DPPH free radical after being exposed to radical scavengers (DPPH radical scavenging activity); measuring the decrease in color intensity of ferrozine-Fe^{2+} complex in presence of chelating agents (Ferrous ions and Fe^{2+} chelating activity); measuring the conversion of Fe^{+3} ions in ferric chloride to Fe^{+2} ions (Reducing power activity) and measuring the color intensity of MDA-TBA complex (Inhibition of lipid peroxidation). The result of AOA of CCF extract as obtained by above mentioned methods is presented in Table 3 and showed concentration dependent AOAs irrespective of the method used suggesting that the flower possesses powerful antioxidant properties. The antioxidant activity of *C. cristata* flowers has been reported previously which clearly support our findings (Kim et al., 2012, 2015; Woo et al., 2011).

The DPPH radical scavenging activity assay is an easy and accurate method for testing the anti-radical activity of a wide range of compounds in a relatively short time. The CCF extract exhibited dose- dependent DPPH radical scavenging ability. The overall percentages of DPPH scavenging activity ranged from 68.7% to 79.9% at different concentrations (100–1000 μg/ml). The ability of CCF extract to quench free radicals may be linked to presence of hydrogen- donating betalain compounds (Cai et al., 2003). This suggests that the flower is a potent free radical scavenger that can be used as a powerful antioxidant in various food systems.

Transition metals are considered to be very active pro-oxidants that catalyze oxidative reactions resulting in ROS generation. Furthermore, these metal ions, particularly ferrous ions, affect the taste and flavor of food systems thereby causing deterioration. The reactivity of such metals can be inhibited by chelators that offer antioxidative effects by eliminating the possibility of endogenous metals to participate in redox reactions. The *in-vitro* ferrous ion chelating activity of samples follows a simple principle of reduction in color intensity of the Ferrozine-Fe^{2+} complex disrupted in presence of metal chelators. The study revealed that metal chelating ability of the extract increased with increasing concentration and was 81.07% at highest concentration (1000 μg/ml). This implies that extract is capable of preventing generation of free radicals and thereby preserving the aesthetic value of food. The metal-binding property may be due to polyphenolic compounds and flavonoids such as kaempferol and quercetin present in the flower. These have been demonstrated to be capable of complexing Fe^{2+} (Fernandez et al., 2002). Oxidation of lipids is an accumulated effect of ROS that attack the lipids and lipid containing molecules such as lipoproteins resulting in their oxidative deterioration. These ROS mostly attack polyunsaturated fatty acids where activated methylene bridges are the critical target sites, generating lipid peroxides. LP results in development of rancidity and off-flavors in fatty foods and oils. The consequences of lipid peroxidation (LP) are severe and are linked to various disorders including cancer and neurodegenerative diseases. The study reveals that the CCF extract inhibited LP in dose- dependent manner ranging from 53.35% to 76.10%. Previous reports suggest that phenolic compounds present in plant extracts are responsible for suppressing LP though various mechanisms (Mathew and Abraham, 2006).

The reducing power of CCF extract as a function of its concentration dependent response relation was found in the reductive ability of the

Table 3. Anti-oxidant activity (AOA) of CCF extract (indigenous variety) evaluated by various *in-vitro* procedures.

Concentration (μg/ml)	RSA_{DPPH} (%)	MCA (%)	LPI %	RPA (Absorbance)
100	68.7 ± 0.13^{a}	44.08 ± 1.93^{a}	53.35 ± 0.43^{a}	0.313 ± 1.34^{a}
200	70.5 ± 1.23^{b}	62.90 ± 0.93^{b}	57.02 ± 0.11^{b}	0.420 ± 0.11^{b}
400	72.4 ± 0.10^{c}	70.10 ± 0.7^{c}	59.95 ± 0.16^{c}	0.673 ± 0.553^{c}
600	75.2 ± 1.73^{d}	77.20 ± 1.21^{d}	71.90 ± 1.22^{d}	0.799 ± 1.34^{d}
1000	79.7 ± 1.83^{e}	81.07 ± 0.23^{e}	76.10 ± 1.23^{e}	0.814 ± 1.22^{e}

Values expressed are mean ± standard deviation.
Different superscripts show significant differences in the antioxidant activities between different extract concentrations ($p < 0.05$).
Where; RSA-DPPH radical scavenging activity.
MCA-metal chelating activity.
LPI-lipid peroxide inhibition.
RPA-reducing power activity.

sample extract. The absorbance increased with increase in concentration of the extract. At the concentration of 1000 μg/ml, the absorbance value was measured to be 0.814. This may be due to the presence of hydrogen donating reductones in the flower (Shimada et al., 1992).

4. Conclusion

This is the first report on the nutritional composition and antioxidant capacity of the *Celosia cristata* inflorescences (red variety) from Kashmir Valley. The results of our present investigation have shown the potential of *C. cristata* as an edible and medicinal plant. The flowers are a source of biologically active and nutraceutical components with important pharmacological and antioxidant effects. Our findings suggest that CCF extract can strongly quench free radicals, inhibit the peroxidation of lipids, and preserve the aesthetics of food by chelating metal ions. In the present investigation, the major anti-oxidant components were phenols, flavonoids and α-tocopherol. Ascorbic acid and secondary metabolites like saponins, and alkaloids found in CCF are also known to exhibit antioxidant activities. This implies that presence of CCF extract in the diet could reduce the risk of several degenerative diseases, depending upon the bioavailability of the compounds. The flower was found to be a good source of minerals, fatty acids and amino acids. As mentioned earlier, *C. cristata* is a potential source of betalain pigment which is responsible for its brilliantly colored inflorescences. The pigment demonstrates high antioxidant and anti-inflammatory properties, suggesting that it may be adopted at a commercial scale for the production of an important food additive with promising health benefits.

Declarations

Author contribution statement

Rukhsaar Sayeed: Performed the experiments; Wrote the paper.

Monika Thakur: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Adil Gani: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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