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Research Article

Isolation, Purification and Quantification of Quercetin and Primary Metabolites from Onion *(Allium cepa L.)*

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Abstract: Plants constitute a key component of drug in fold system, and thus are a source of inspiration for many drugs. This study was conducted to quantify carbohydrates, proteins, lipids, metal ions and flavonoids in the bulbs of three different varieties of *Allium cepa* L. Gas chromatography (GC) analysis was performed for analysing fatty acids while high-performance liquid chromatography (HPLC) was employed for quantification of Quercetin. Results of this investigation revealed that dark pink *A. cepa* contains higher concentration of cellulose (17.55%) and lower concentration of oil (2.12 %) and its GC analysis showed that propionate concentration was much higher (31.61 %). Levels of protein and crude fat in the onion samples were 8.75% and 9.9%, respectively. Macro elements were observed in high concentration and sodium was highest (14.0 µg/mL); however, copper concentration was observed to be in traces (0.3 µg/ mL). The HPLC analysis of flavonoids revealed that A. cepa was rich in Quercetin-3, 4 - O-diglucoside (3, 4- Qdg); Quercetin-3-O-glucoside (3-Qmg or iso quercitrin); Quercetin-4 -O-glucoside (4-Qmg); Isorhamnetin-3-O-glucoside (3-Img), Quercetin aglycone (Q); and Kaempferol (K) in which concentration of Quercetin-4 - O-glucoside (4 - Qmg) was significantly higher. The present study helped in purification of fatty acids and quercetin and for formulation of drugs which utilize these phytochemicals.

Keywords: *Allium cepa* L, phytochemicals, HPLC and GC analysis

1. INTRODUCTION

Approximately fifty thousand higher plant species, in which about one in sixth of all species are in practice as medicinal plants which represents so far the largest consumption of natural world. Many species are in practice only in traditional medicine systems (only 50,000). About hundred species of plants have added meaningfully to contemporary medicines and usage of medicinal plants has been increased throughout the world, linked to the perseverance and occasionally growth of folk drug and an increased concern in plant treatment. Fruits and vegetables are source of dietary vitamins, minerals, and fiber and are a chief source of flavonoids in foods. Epidemiological readings says that flavonoids defend against cardiovascular disease and, to a smaller extent, against cancer and other age related disorders, e.g., dementia [12].

The phytochemical which is found in abundance in onions is quercetin that is a member of the flavonoid family which is called flavonols. Quercetin prevent bone resorption [15] and is useful to work under microgravity works as such long term mission leads to depletion of calcium (Ca) resulting in decrease in bone density [13]. Few onion varieties are described to comprise plentiful heights of flavonols. Partial research has been done on phytochemical aspects of hydroponically grown crops, including onions. Quercetin has capacity to become a chemotherapeutics agent to cure prostate cancer as described by Xing et al. [17] and quercetin and phenols also have antioxidative activities [15]. New *A. cepa* L. bulbs contain mostly H_2O (about 88%), fat (9.34 g). sugar (4.24 g), Dietary fiber (1.7

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g), fat (0.1 g), saccharides (6 %), energy (40 Kcal or 170 KJ), proteins (about 1.1 g) and flavonoids (22.6 mg) in 100 g of raw material. The specific conformation hinge on many factors, such as growing situations, harvest time, length and storage conditions. Onions can be grown from seed or sets, which were produced by propagating seeds for 01 year, which resulted in undersized plants which are stress-free to set out and grow into matured bulbs later on. These bulbs are less durable than onions which are grown directly [5].

The HPLC system coupled with colorimetric detection of antioxidant activity was developed in order to separate antioxidants, especially the flavoniods, rutin and quercetin and to determine their activities in single step [9]. In the system developed, elute from the HPLC column was split in to two flows at 8:2 ratio. The major part flowed to the UV detector set at 220 nm and mixed the minor part with a free radical, DPPH, to perform a color reaction with the eluted antioxidants. This reaction occurred in a knitted shape reactor (70 cm 08 1.1 PTFE). The peaks indicating antioxidant behavior were checked from the decrease of absorbance at 515 nm. The results showed that the on line HPLC coupled with antioxidant activity detection developed could separate the flavonoids, rutins and quercetin in plant extracts such as Sophora japonica and Morus alba and simultaneously determined their antioxidant activity. The detection limits for the antioxidant activity determination of these two compounds were 500 and 200 mg, respectively. This method could be applied for rapid determination of rutin and quercetin in complex mixtures. Keeping in view the importance of quercetin (flavonoids), present study has been done to gain following aims and objectives

1. To asses level of quercetin in onion bark; and
2. To evaluate onion bark for protein, carbohydrates, essential oils and metal ions concentration.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Samples

2.1.1 Collection of Sample

Three onion samples (Dark pink, White, Green) were purchased from native market of Rawalpindi in fine polythene bags which were already labeled with number and date of collection of sample and saved at 20^{o} C for more experiments.

2.1.2 Preparation of Sample

Bark of onion was separated from roots and leaves. Fresh Samples were sun dried followed by oven drying at 105^{o} C for 12 hours and then crumpled into 80 mesh powder and kept at 4 ^{o}C for advance processes.

2.2 Biochemical Analysis of *Allium cepa* L.

2.2.1 Carbohydrate Analysis:

The acid hydrolysis method of Knekt et al. [8] has been used for carbohydrate analysis. Treated the dried samples were mixed with 1.5 mL of 72% H_2SO_4 in 04 different Pyrex tubes, in a water bath. After 1 hour reaction mixtures was diluted with 42.00 mL of H_2O for 1st 02 tubes and 43.00 mL with other tubes. About 1 mL spiked solution (33 g/1 xylose) was further added to first two tubes and autoclaved for one hour at 121^{o}C. Residues obtained after cooling were examined by HPLC, Shamizdu, 1200 System prepared with Amines hpz, 87 h organic acid analysis column (Bio, Rad) at 60^{o}C. The eluent was 4 mM sulphuric acid with flow rate of 0.6 ml / minutes exposure on a RI detector. Before HPLC analysis, 10 µL of H_2SO_4 was added in 1.00 mL samples, centrifuged at 14,000 rpm monitored by separation with 0.45 µm membrane/ minute for 10min.

2.2.2 Analysis of Protein:

Micro Kjeldahl nitrogen analysis was used to determine protein concentration by applying AOAC 969.19 and 921.89 method [1] and Lowry's method [11].

2.2.3 Lipid Analysis

This analysis was done by AOAC method, 921.85 by using soxhlet apparatus in which 5.0 g of 80 mesh sample was filled in thimble to extract oils by using diethyl either for 08 hours [1].

2.2.3.1 Analysis of fatty acids: The lipids present

in onion sample were analyzed after they were diversified with Boron tri fluoride (BF3) in CH_3OH which converted fatty acids into tri- methyl-ester derivatives using method of Elmuez [6]. These esters were thawed in CHCl3 and examined with the help of gas chromatography.

2.2.3.2 Gas Chromatographic (GC) analysis: Gas chromatography analysis was done on an Agilent 6890 N Network GC system. HP Innowax. Capillary column of 60.0 mm was used. Temperature was raised up to 185°C with 10oC for 3 minutes after insertion and raised up to 185 °C / minute for 1 min, raised to 200 °C with five minute heating. Final temperature was raised to 220 °C / minute heating for 20 min. Injector temperature was kept at 250 °C with FID detector 275 °C. Carrier gas was Helium with 40.65 psi inlet pressure; 39 cm / s of linear gas velocity; 2.7 mL / min of column flow rate, split ratio of 40 and injected volume of 1 µl.

2.2.4 Determination of Metal Ion

For metal ion detection, samples have been digested by dry digestion method [16] in which 01 gram sample was kept in porcelain crucible to ash it at 450 °C for 18 to 20 hours. Then dissolved the ash and blank in one mL. concentrated HNO_3 and vaporized for desiccation and again heated at 450 °C for four hours and then treated with one mL H_2SO_4 (conc.), one mL nitric acid, one mL hydrogen peroxide which was diluted with deionized H_2O up to 50 mL. Ca, Mg, Zn, Fe and Cu of sample were analyzed by atomic absorption spectrophotometry while Na and K by flame atomic absorption spectroscopy.

2.2.5 Determination of Flavonoid

Flavonoid concentration was measured by mixing five grams sample in fifty mL. of 80 % aqueous C_2H_5OH for one day and then centrifugation at 10,000 rpm at room temperature for fifteen minutes. Then the pallet was rejected and the supernatant saved, which was supposed to contain flavonoid, at 4 °C. Calorimetric assay was done according to the protocol of Lillian et al. [11] for flavonoid estimation. 250 µl of flavonoid extract was assorted with 1.25 mL. distilled H_2O and 675 µl of 5.0 % sodium nitrite and after five minutes, 150 µl of 10% hydrated aluminum chloride solution was added. After six minutes 500 µL of one molar sodium hydroxide and 275 µl of distilled H_2O were also mixed. After well mixing of solution, absorbance was read at 415 nm. Standard was changed dilutions of Quercetin (50 to 250 µg).

2.2.6 Isolation and Quantification of Quercetin

2.5.6.1 Column chromatography for isolation of quercetin: Liquid column chromatography was done with silica gel of 70- 230 mesh size with 80% methanol. Total 12 elutions were collected through this column. The 4th and 5th elutions were combined and were again run through liquid column in which silica gel of 230- 400 mesh size was used while solvent system used in this column was mixture of $C_4H_{10}O$; CH_3COOH; H_2O (4:1:5). Elutions obtained from this column were saved for HPLC analysis.

2.2.6.2 Sample Preparation for HPLC Analysis: About 50 powdered sample of onion has been dissolved in 500 mL of 80% ethanol and left for shaking at 25 °C for 24 hours and shaking and centrifuged at 10 oC for 10 minutes at 10000 rpm. The mixture was filtered using Whatman filter paper No. 41 and the filtrate was left at room temperature so that the solvent gets evaporated up to dryness. The sample was then stored at -20 °C for further use in column chromatography.

2.2.6.3 HPLC Analysis of Quercetin: It has been done as described by Patil et al. [13]. Twenty grams of onion sample, by removing dry leaves, was grounded with 80 mL C_2H_5OH (80%), for one minute, filtered and stored at minus 20 °C till analysis. 5 mL aliquot of stored extract was dried under vacuum at room temperature and re-suspended in one mL C_2H_5OH (80 %). Then extract was filtered through 0.45 um nylon 66 filter paper and injected 10 µL from this solution into the HPLC system. Perkin-Elmer model of HPLC with binary LC pump of 250, an LC 600 auto sampler, a UV/V with spectrometric detector of LC- 290, PV Nelson 900 series INTERFACE, Hewlett- Hewlett-Pakkrad 3394 Integration and a Bonda pak C-18 column (250*4.6 mm). Mobile phase was 0.5% orthophosphoric acid in CH_3OH; 1 mL/ minute flow rate was set and Aglycone content was measured using Quercetin dihydrate 95% as standard.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of *A. cepa* L.

According to chemical composition analysis, dark pink *A. cepa* shows higher concentration of cellulose (17.55%) and lower concentration of oil (2.12 %). Although higher concentration of volatile oil in onions (16 %) has been reported but the lower concentration of oil from onion found in this study was due to heating of reaction mixture at temperature, (40-60 ^{o}C) up to 18 hours. It was assessed that prolong affected on oil quantity resulted in degradation of polyunsaturated fatty acid [7]. The level of protein (8.75 %) and crude fat (9.9 %) was found in onion samples (Table 1 and Fig. 1). Although reported concentration of protein in onion is 1.1g/ g of onion [4]. Because of more protein and low fat content in onion have made it beneficial food which can be used as healthy medicinal food especially for persons suffering from cardiovascular diseases.

3.2 GC analysis of *A. cepa* L.

The essential oil extracted from dark pink onion samples has been further fractionated into its components by GC analysis (Table 2 and Fig. 2). Higher concentration of propionate (31.61 %) was found in oil obtained from onion samples. So it can be said that higher concentration of propionate is responsible for antimicrobial and antoxidant properties of Allium cepa. Acetate, isobutyrate and butyrate are absent whereas significant concentration of isovalerate (6.42 %) and valerate (6.56 %) were present in onion samples which indicates that onion oil is basically essential oil and could be used for preparation of therapeutic agents.

3.3 Identification and Quantification of Quercetin

The mixtures of acetonitrile with CH_3COOH, orthophosphoric acid and $KHPO_4$, in many concentrations and used proportions by using variant technique of optimization to make a diverse configuration of mobile phase to achieve maximum separation of quercetin in a relatively small period of time. Better results were achieved by using methanol with O-phosphoric acid (90:10), with reference to separation efficiency and sensitivity. Use of O-phosphoric acid in the solvent system decreased pH and gave better separation of phenolics. Moreover, addition of different concentration of O-H_3PO_4 in solvent system were checked to finalize its optimal condition which was compromised among column stability, separation competence and sensitivity of determination. Deceptive pH for 0.05 % O-phosphoric acid was finally acidic. Retention times of investigative method depended on mobile phase. The retention time (22.767 min) was used for quercetin analysis from onion samples as compared to 3.058 min used for standard quercetin analysis.

Retention times and UV spectra of peaks were compared with reliable standard (Table 3 and Fig. 3 and 4). Fig. 3 shows the amount of quercetin in different forms in one sample of onion while Fig. 4 shows amount of quercetin in onion at its different growth stages. For checking the linearity of the relationship between peak areas and quantity of standard, a concentrated standard was used and diluted with 80% CH_3OH. Concentration of standard loaded in HPLC system was 1 mg/ mL and (10 µl) of sample. The Limit of detection (LOD) of quercetin was 0.5 ug/ mL, determined by UV detector at 374 nm and calculation of detection limits for compound was based on a signal- noise ratio of quercetin during experiment.

Limit of quantification (LOQ) was 140 ng/ mL for quercetin and results obtained for LOQ of quercetin confirmed that the used HPLC method was much sensitive for determination of quercetin in this type of sample. The results of quercetin quantification are shown in table 3 and Fig. 3 and 4. The results revealed that the sample of onion contained much higher concentration of quercetin in onion sample which was presented by high peak obtained by HPLC analysis (Fig. 3) Quercetin dihydrate (standard) analyzed from Shamizdu and compared to quercetin analyzed from onion sample (Fig. 4) These results that are obtained in present study provided much lower concentration of quercetin from onion as compared to the results reported by Patil et al. [13] who reported that this variation is because of different location, growth stage and type of soil in which onion was grown.

Table 1. Chemical analysis of *Allium cepa* L (Dark Pink).

Sr. No.	Chemical component	Amount (%)
1	Total fat	9.9 ± 0.5
2	Total protein	8.75 ± 0.42
3	Total cellulose	17.55 ± 1.9
4	Total hemicellulose	7.135 ± 1.02
5	Total volatile oil	2.12 ± 0.71
6	Total flavonoids	1.75 mg/g of sample

± = Results obtained after triplicate analysis

Table 2. Various Oils extracted by Gas Chromatography from dark pink *Allium cepa* L.

Sr. No.	Fatty acids	Amount (%)
1	Acetate	2.5 ± 0.13
2	Propionate	31.61 ± 2.9
3	Butyrate	2.5 ± 0.19
4	Isobutyrate	2.5 ± 0.15
5	Valerate	6.56 ± 1.02
6	Isovalerate	6.42 ± 1.0

± = Results obtained after triplicate analysis

Table 3. Concentration of quercetin in onion, spectrophotometric and HPLC (362 nm) analysis.

3,4'-quercetin-O-diglucoside (a)	4'-quercetin-O-glucoside (b)	c= a + b ($mg \cdot kg^{-1}$ fwt)	% TF= c × 100	d= Total quercetin (mg/ kg fwt)	% TF= D × 100	TF *
113.7 ± 13.1	137.7 ± 18.0	251.3 ± 30.6	88%	253.6 c ± 30.9	89%	285.5 c ± 33.5
125.1 ± 9.8	149.4 ± 15.1	274.6 ± 24.2	90%	278.2 c ± 24.9	91%	306.3 c ± 26.5
134.9 ± 9.3	241.6 ± 26.0	376.5 ± 35.1	84%	399.0 b ± 44.9	88%	453.9 b ± 48.3
211.1 ± 16.0	267.5 ± 17.0	478.6 ± 30.5	93%	481.0 ab ± 30.7	93%	516.4 ab ± 33.6
202.2 ± 12.8	300.1 ± 23.5	502.3 ± 36.0	86%	513.3 a ± 37.3	88%	580.9 a ± 42.4

Table 4. Metal Ions in bulb of dark pink *A. cepa* L.

Metal Ions in A.cepa	Microelements				Macroelements			
	Zinc	Iron	Manganese	Copper	Magnesium	Calcium	Sodium	Potassium
Concentration (µg/ml)	0.34±0.07	0.863±0.04	0.2±0.01	0.3±0.02	13.276±1.06	0.864±0.01	14±1.05	9.25±3.05

± = Results obtained after triplicate analysis

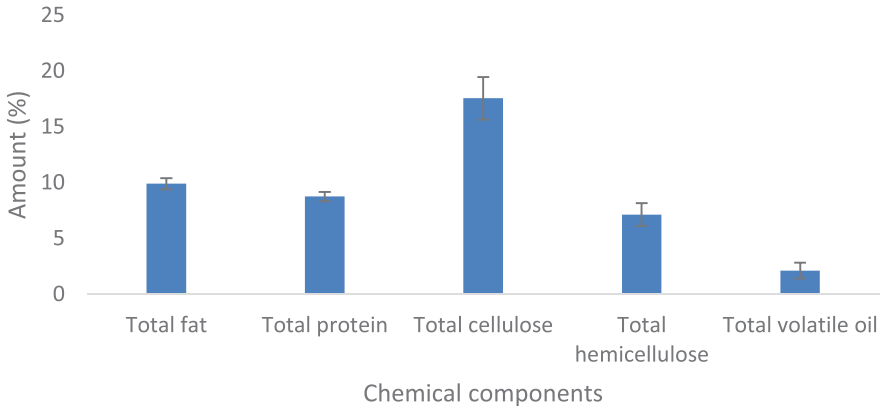

Fig. 1. Chemical analysis of *Allium cepa* L. (dark pink).

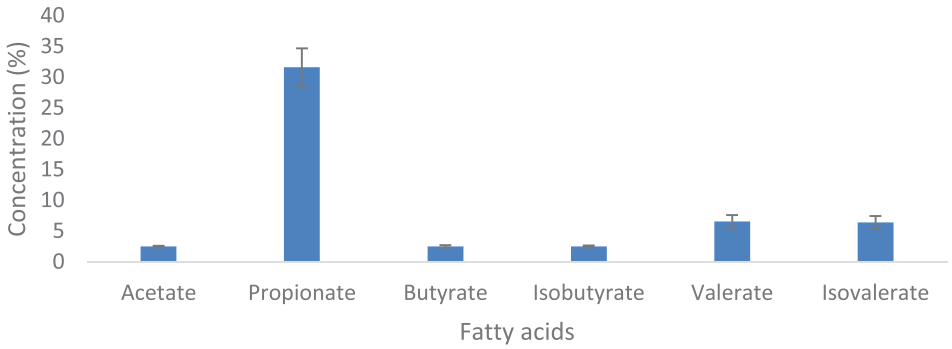

Fig. 2. Various Oils extracted by Gas Chromatography from dark pink *Allium cepa* L.

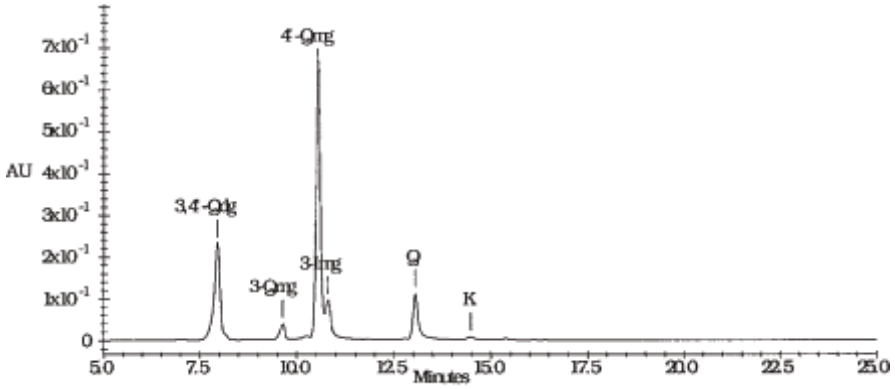

Fig. 3. HPLC chromatogram of C2H5OH extract of dark pink onion at 362 nm eluted from 5.0 to 25.0 min. L to R: Quercetin-3,4'-O-diglucoside (3,4-Qdg); quercetin-3-O-glucoside (3-Qmg or isoquercitrin); quercetin-4'-O-glucoside (4'-Qmg); isorhamnetin-3-O-glucoside (3-Img), quercetin aglycone (Q); and kaempferol (K).

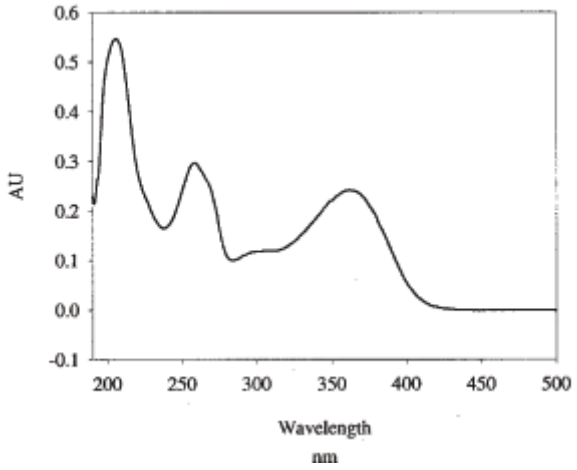

Fig. 4. Absorbance spectra at190–500 nm, of iso quercitrin (quercetin-3-O-glucoside), standard in 80% Ethyl alcohol. Maximum absorbance of isoquercitrin occurred at 258 nm in band II and 362 nm in band I.

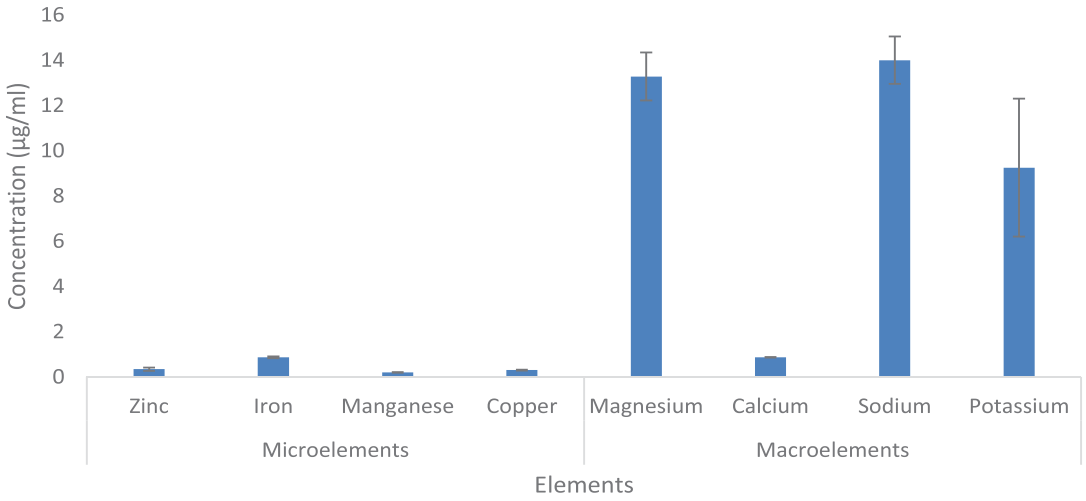

Fig. 5. Metal Ions in dark pink *A. cepa* L.

Allium species are a best source of phytonutrients and are used to treat or prevent a number of ailments, e.g., cancer, coronary heart disease, obesity. Hypercholesterolemia, diabetes type 2, hypertension, cataract and disturbances of the gastrointestinal tract (e.g., colic pain, flatulent colic and dyspepsia).

3.4 Analysis of Metal ion from Onion Samples

Calcium, magnesium, zinc, iron and copper in *A. cepa* were analyzed by atomic absorption spectroscopy (AAS) and sodium and potassium were determined by flame photometry (Table 4, Fig. 5). Macro elements including magnesium (13.276 µg/mL), sodium (14.0 µg/mL) and potassium (9.25 µg/mL) were found in higher concentration while microelements were found only in trace amounts. The concentration level of calcium (0.864 µg/mL) and iron (0.863 µg/ml) were found in equal amount while manganese (0.2) µg/mL) and zinc (0.340 µg/mL) were found in less amounts. It was observed that concentration of Zn was found in this study was different from that reported by Benkeblia et al. [2] after analysis of onion samples. Whereas concentration of Co and Ni found in onion samples were lower than concentration of these metal ions reported by Zoltan et al. [18]. Nutritional value of *A. cepa* increases due to metals. However, it is important to notice that metal ion configuration of onion chiefly depends on ecology and substrate arrangement [3] as onions have more capacity to remove metal ions from substrate while most of them are cofactor for many enzymes, e.g., zinc and magnesium trigger DNA polymerase and magnesium controls many enzymes of glycolysis. *A. cepa* is also a rich source of iron which is an important component of hemoglobin and thus helps in transport of oxygen in body as was reported by Hansen [6]. Sodium and potassium are very important metal ions for controlling blood pressure inside the body. Potassium deficient diet may raises blood pressure in normal and healthy persons while food rich in potassium may lower blood pressure in hypertensive patients. *A. cepa* contains higher concentration of potassium and lower concentration of sodium and therefore can be considered a good food for hypertensive patients. However many reports indicate the presence of trace amounts of calcium in *A. cepa* that decreases its nutritive value for calcium deficient persons as it may lead to osteoporosis.

4. CONCLUSIONS

Plants are main source of many components for the formulation of several drugs by the pharmaceutical industry. This research was conducted to quantify concentrations of primary metabolites and metal ions in various varieties of *Allium cepa* L. This study revealed that dark pink *A. cepa* has more cellulose than oil, and concentration of propionate was highest. Macro elements were higher in concentration than micro elements. The HPLC analysis of flavonoids revealed that *A. cepa* is rich source of Quercetin-3, 4 - O-diglucoside (3, 4- Qdg); Quercetin-3- O-glucoside (3-Qmg or iso quercitrin); Quercetin-4 -O-glucoside (4

- Qmg); Isorhamnetin-3-O-glucoside (3-Img), Quercetin aglycone (Q) and Kaempferol (K), in which Quercetin-4 - O-glucoside (4 - Qmg) was highest in concentration. Thus, purified quercetin derivatives from onion can be used beneficially in the pharmaceutical industry.

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