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PHYTOCHEMICAL STUDIES ON THE METHANOLIC EXTRACT OF *CORIANDER SATIVUM* LEAVES – AN *IN VITRO* APPROACH

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ABSTRACT
Coriander belongs to the family Apiaceae and has great medicinal use. It is used to treat Gastro-intestinal disorders such as Anorexia, Diarrhea, pain, vomiting, Urethritis, Cystitis, Urinary tract infections, Urticaria, rashes and burns. The present study was formulated to quantify the concentration of selected phytochemicals that are present in the methanolic extract of *Coriander sativum* leaves using spectrophotometric and HPLC method. It was found the total amount of carbohydrates and proteins was found to be mostly in the form of glycosides. The Polyphenols present in *Coriander sativum* leaves are vanillic, p-coumaric, cisferulic and trans-ferulic acids which are responsible for their antioxidant activity.

KEYWORDS: HPLC, Polyphenols, spectrophotometer, antioxidant.

1. INTRODUCTION

Coriander sativum is a main habitat in Russia, Central Europe, Asia and Morocco.[1] Coriander is usually cultivated in winter or summers and mainly in Black soil.[2] Coriander is a native plant of Eastern Mediterranean region from where it may have spread to India, China and rest of the world. Coriander leaves are rich in moisture i.e they constitute about 87.9% moisture; 3.3% protein; 6.5% carbohydrates and 1.7% total ash.[3]

Figure 1: *Coriander sativum*.

Taxonomical Classification

Kingdom	:	*Plantae*
Subkingdom	:	*Tracheobionta*
Division	:	*Magnoliophyta*
Class	:	*Magnoliopsida*
Subclass	:	*Rosidae*
Order	:	*Apiales*
Family	:	*Apiaceae*
Genus	:	*Coriandrum*
Species	:	*Coriandrum sativum*

Coriander belongs to the family Apiaceae and has great medicinal use. This plant is a potential source of lipids and essential oils.[4] The young plant (green coriander) is used in preparing sauces and flavouring of curries and soups. The stem leaves and seeds have pleasant aroma. Coriander seed oil is an aromatic stimulant, an appetizer and a digestant stimulating the stomach and intestines. It is beneficial to nervous system. Coriander is mainly used in masking foul medicines.[5] Coriander oil is used in cosmetics, body care products and perfume. It has certain therapeutic applications: coriander seed are used to treat Gastro-intestinal disorders such as Anorexia, Diarrhea, Pain and vomiting.[6] It is useful in Urethritis, Cystitis, Urinary tract infections, Urticaria, rashes and burns. The coriander species have certain nutritional constituents like water, carbohydrates, proteins, fat, calcium, potassium, sodium, phosphorous, iron, and Vitamin A. Phytochemical and anti-microbial effect is mainly from leaves and seeds. This medicinal plant serves as a source of drugs. Fresh juice of coriander helps in curing many deficiencies related to vitamins and iron.[7]

In addition to vitamins, pro-vitamins and minerals, presence of phytochemicals in fruits and vegetables is considered to be nutritionally important in the prevention of certain chronic diseases like cancer, diabetes and cardio-vascular diseases.[8] The most widely used components of the coriander plant are seed with most important constituents like essential oils and fatty oils. The fatty oils are around 25% of seed. Essential oils are about 1%. Fatty oil is light yellow in colour and has a characteristic smell. The oil contains high amount of Petroselinic acid, which makes it unique. High levels of glycolipids are found in seed oil including Acylatedsterylglucoside, Sterylglucoside and Glucocerebroside. Over few last decades the essential oil has gained popularity as a source of bioactive with many health benefits. The component of essential oil appears to be dependent on biological and geographical variabilities.[9] The essential oil content of ripe and dried seeds of coriander varies between 0.03% and 2.6%. The major component of which is S-(+)-linalool (60-70%), other minor components present in essential oils are monoterpenes – hydrocarbon.[10]

This study was formulated to quantify the concentration of selected phytochemicals that are present in the methanolic extract of *Coriander sativum* leaves using spectrophotometric and HPLC method.

2. MATERIALS AND METHODOLOGY

Preparation of the *Coriander sativum* leaves methanolic extract

10 grams of the dried *Coriander sativum* leaf material was powdered and placed in Soxhlet extractor along with 150 ml of methanol and refluxed at 60°C for 8hrs. The methanolic extracts were filtered through Whatmann No. 1 filter. The filtrate was evaporated to dryness at 80°C and stored until further analysis.

2.1. Phytochemical analysis

A small amount of the methanol extract was used for the phytochemical analysis. The phytochemical tests include the test for alkaloids, flavonoids, tannins and phenols, saponins, steroids, terpenoids, carbohydrates, amino acids and proteins.

Test for alkaloids

To 1 ml of extract, few drops of Mayer's reagent were added and the result was observed. To 1 ml of extract, few drops of Wagner's reagent were added and the result was observed.

Test for flavonoids

To 1 ml of extract, few drops HCl was added and heated for few minutes. Then few drops of Sodium Hydroxide were added and the result was observed.

Test for tannins and phenols

To 2 ml of extract, 1-2 drops of dilute ferric chloride and 10% of lead acetate was added. The result was observed.

Test for saponins

To 3 ml of aqueous extract, 10 ml of distilled water is added. The solution is shaken vigorously for 5 mins. It is allowed to stand for about 30 mins and the result was observed.

Test for steroids

To 1 ml of the extract, add 2 ml acetic anhydride and 2 ml of conc. H_2SO_4. Mix well. Observe for color change.

Test for terpenoids

Salkowski's test- To 1 ml of the extract, add 2 ml of chloroform and 3 ml of conc. H_2SO_4. Observe for the colour change.

Test for carbohydrates

To 1 ml of extract, few drops of Benedict's solution was added and then heated. The result was observed.

Test for amino acids

To 1 ml of the extract, add 10% of Ninhydrin solution. Mix well. Observe for colour change.

Test for proteins

To 1 ml of the extract, add few drops of FC reagent. Mix well. Observe for colour change.

2.2. Estimation of Carbohydrates by Ortho-Toluidine Method

Glucose condenses with ortho-toludine in glacial acetic acid when heated to 100°C. The product formed is N-Glycosylamine which was blue green in colour, the absorbance which is measured at 630 nm.

2.3. Estimation of Protein by Lowry's Method

Protein reacts with Folin-Ciocalteau reagent to give a coloured complex. The colour so formed was due to the reaction of alkaline copper protein and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of the colour depends on the amount of these aromatic amino acids and measured using 660 nm.

2.4. Estimation of Total Phenols by FC Method:

The colorimetric method is the most widely used method for the estimation of total phenolic content. The reagent used for this estimation is the Folin-Ciocalteu reagent, which is a mixture of phosphomolybdate and phosphotungstate. This method consists of calibrating using the standard phenolic compounds. FC reagent reacts with the nitrogen-containing compounds to form a blue coloured complex. The intensity of the color was read at 650 nm.

2.5. Estimation of total Polyphenols by High Performance Liquid Chromatography (HPLC)

Plant Extraction

10gms *Coriander sativum* powder was extracted with 150 ml methanol at 50°C for 4 hours. The methanolic extract of *Coriander* leaves were filtered through

Whatmann No. 1 filter paper and filtrate was evaporated to dryness. Methanolic extract (10mg/ml) was used for HPLC analysis. The standard gallic acid (2.5µg/mL) and *Coriander* leaves methanolic extract (3.57µg/mL) were dissolved in 1mL of mobile phase and 20µL was injected and the elution was monitored at 310nm.

HPLC Condition:
Instrument : Shimadzhu LC- Prominence 20AT
Column : C18 column 250 mm x 4.6 mm, 5u particle
Mobile Phase : Linear
A : HPLC grade Acetonitrile (70%)
B : HPLC grade water (30%)
Flow Rate : 1.0 ml / min
Injection volume: 20ul

Quantification of polyphenol in extracts
Concentration of Standard injected: 2.5µg/ml
Sample concentration : 3.57 µg/ml

2.6. Estimation of antioxidant property by FRAP assay

Ferric reducing antioxidant power (FRAP) is a widely used method to determine the antioxidant capacity of the samples. This method uses antioxidants as reductants in a redox-linked colorimetric reaction in which ferric (Fe^{3+}) is reduced to ferrous (Fe^{2+}). The reduction of ferric to ferrous at low pH leads to the formation of a coloured ferrous-probe complex from a colourless ferric-probe complex. The intensity of the colour was read at 593 nm using colorimeter.

2.7. Estimation of Reduced Glutathione (GSH)

The Reduced Glutathione content in the sample was estimated by the method of Thomas et al., 1985. 0.2 ml of sample was made upto 1.0 ml by addition of 5% TCA and the protein in the sample was precipitated by centrifugation. 0.2 ml of the protein free supernatant was used for the assay. 2.0 ml of DTNB was mixed with 0.2 ml of the supernatant and the final volume was made up to 3.0 ml with phosphate buffer and the optical density was measured at 412 nm in a spectrophotometer within 60 seconds, against the blank. The blank contained 0.2 ml of TCA and 2.0 ml of DTNB, which was made upto 3.0 ml with phosphate buffer. The standard glutathione was prepared in separate tubes at a concentration range of 5 to 20 µg were treated with 2.0 ml of DTNB and the volume was made upto 3.0 ml with phosphate buffer. The blank and the standard were also measured at 412 nm. The amount of reduced Glutathione in the sample was expressed in mg / dl.

3. RESULTS

3.1. Phytochemical analysis

The observation for qualitative phytochemical analysis on methanolic extract of *Coriander sativum* leaves are shown in the Table – 1.

Table – 1: Qualitative analysis for phytochemicals present in methanolic extracts of *Coriander sativum* leaves.

Phytochemicals	Test	Observations	Inference
Alkaloids	Mayer's Test Wagner's Test	No Orange coloured precipitate No Reddish-brown coloured precipitate	Absence of Alkaloids
Flavonoids	2 M HCl + Aqueous NaOH	Yellow colour is observed	Absence of Flavonoids
Tannins and Phenols	10% Lead Acetate + $FeCl_3$	Brick red colour is observed at top layer of the test tube White colour is observed at the bottom of the test tube	Presence of Tannins and Phenols
Saponins	Foam Test	No Foam is appeared	Absence of Saponins
Steroids	Acetic Anhydride	No Violet to Blue or Green	Absence of Steroids
Terpenoids	Salkowski's Test	Presence of Reddish Brown colour	Presence of Terpenoids
Carbohydrate	Benedict's test	Red colour precipitate is observed	Presence of Carbohydrates
Amino Acids	Ninhydrin Test	Purple Colour	Presence of Amino Acids
Proteins	FC Reagent	Green Colour	Presence of Proteins

3.2. Estimation of Carbohydrates by Ortho-Toluidine Method

The total amount of carbohydrates present in the methanolic extract of *Coriander sativum* leaves was found to be 37µg/ml.

3.3. Estimation of Protein by Lowry's Method

The total amount of proteins present in the methanolic extract of *Coriander sativum* leaves was found to be 120 µg /ml.

3.4. Estimation of total phenols by F C Method using spectrophotometer

The total amount of phenols present in the methanolic extract of *Coriander sativum* leaves was found to be 290 µg / ml.

3.5. Estimation of total phenols by HPLC Method

The chromatogram of gallic acid standard and Poly Phenol Overlay present in *Coriander sativum* leaves extract are shown in the Figure – 3 and Figure – 4. Using the area and height compared with standard the Polyphenol concentration present in *Coriander sativum* leaves extract can be calculated.

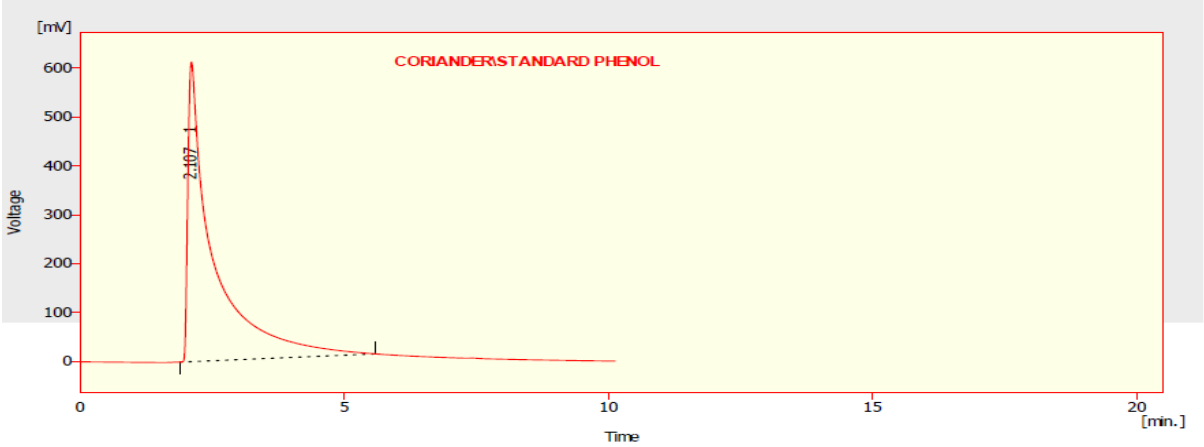

Figure – 2: HPLC chromatogram of Standard Phenol using Gallic acid.

Table – 2: HPLC chromatogram of Standard phenol using Gallic Acid.

	Retention Time (Min)	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.107	20441. 145	611.849	100.0	100.0	0.31
Total		20441. 145	611.849	100.0	100.0	0.31

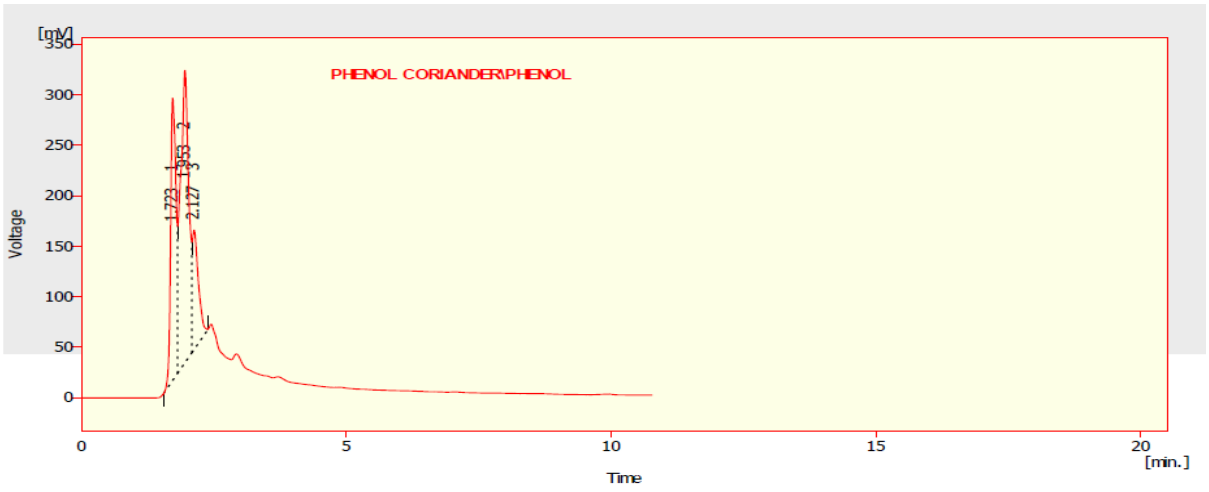

Figure – 3: HPLC chromatogram of Poly Phenol Overlay present in *Coriander sativum* leaves extract.

Table – 3: HPLC chromatogram of Poly Phenol Overlay present in *Coriander sativum* leaves extract extract.

	Retention Time (Min)	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.723	1950.170	279.262	31.9	40.7	0.14
2	1.953	3204.499	289.427	52.4	42.1	0.22
3	2.127	956.486	118.285	15.7	17.2	0.14
Total		6111.155	686.974	100.0	100.0	

The total amount of phenols present in the methanolic extract of *Coriander sativum* leaves was found to be 320 µg /ml.

3.6. Estimation of antioxidant property by FRAP assay

The total amount of antioxidants present in the methanolic extract of *Coriander sativum* leaves was found to be 480 µg / ml.

3.7. Estimation of Reduced Glutathione (GSH)

The total amount of reduced Glutathione present in the methanolic extract of *Coriander sativum* leaves was found to be 40 µg / ml.

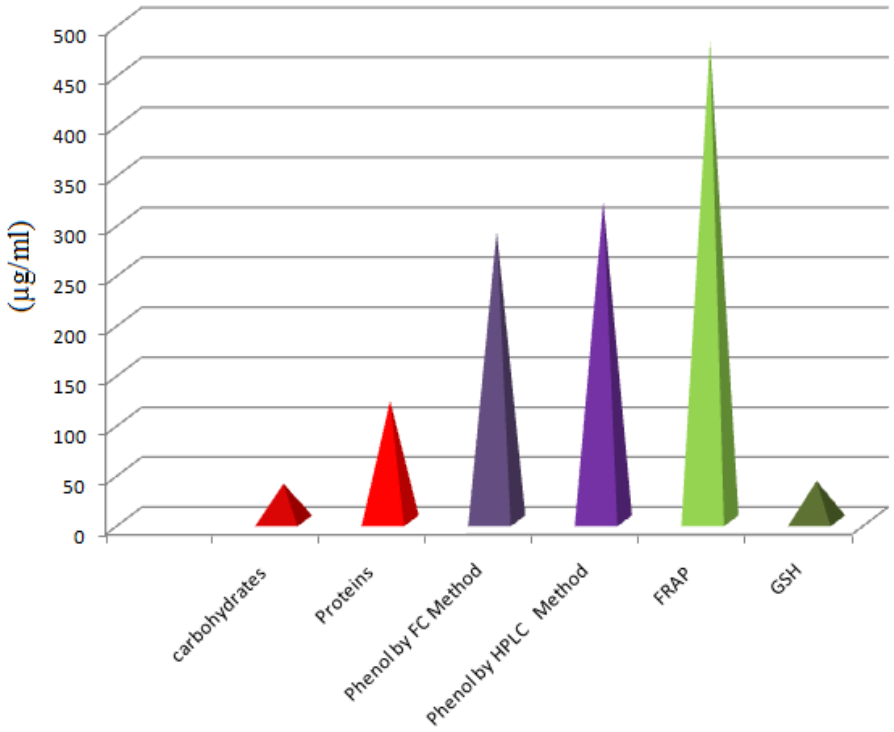

Figure – 4: Comparison of conc. of Carbohydrates, proteins, phenols, FRAP and GSH in methanolic extract of *Coriander sativum* leaves.

4. DISCUSSION

4.1. Phytochemical analysis in the methanolic extract of *Coriander sativum* leaves

The above Table – 1 represents the phytochemical screening of *Coriander sativum* with methanol. By the preliminary phytochemical test conducted for *Coriander sativum*, the methanol extract showed the presence of Tannins, phenols, terpenoids, carbohydrates, amino acids and proteins but the absence of alkaloids, flavonoids, saponins and steroids.

Tannins are group of polyphenolic compounds which binds to other proteins to inhibit their activity in case of digestive enzymes. These have two subgroups; it can either be condensed form or hydrolyzable form. It can also form cross-linkages between proteins and other macromolecules.[11]

Phenols are widely distributed in plant tissues. They contribute colour, flavor, astrigency to fruits. Its concentration may vary from 0.5 to 5.0g/100g dry weight of plant tissues. These phenolic compounds are considered as secondary metabolites of plant metabolism which contributes to physiological or ecological functions. Phenolic groups may be categorized into four main groups depending upon number of phenol rings and structural elements that bind these rings. These groups include: flavonoids (anthocyanins, flavones and Iso - flavones) tannins, stilbenes and lignans.[12]

Terpenoids are also known as isoprenoids. These are most numerous and structurally diverse natural products found in many plants. They represent widespread group of natural products and can be found in all classes of living things. They fight against cancer, malaria, inflammation and variety of infectious diseases.[13]

4.2. Carbohydrates and protein concentration in the methanolic extract of *Coriander sativum* leaves

From the Figure – 4, the total amount of carbohydrates and proteins present in the methanolic extract of *Coriander sativum* leaves was found to be 37 µg/ml and 120 µg/ml. The Carbohydrates and proteins present in the *Coriander sativum* leaves in the form of glycosides. The glycosides consist of various categories of secondary metabolites that are bound to a mono- or oligosaccharide or to uronic acid. There are five main groups of glycosides. They are cardiac glycosides, cyanogenic glycosides, glucosinolates, saponins and anthraquinone glycosides. On ingestion, the glycosides get hydrolyse in the colon, and the more hydrophobic aglycone might be absorbed. The cyanogenic glycosides have aglycones derived from amino acids and these compounds interfere in the iodine metabolism and may results in hypothyroidism. The glucosinolates contain sulphur-

containing amino acid-derived aglycones. These compounds effects on cytochrome P450 isoforms and decrease hepatic bioactivation of environmental procarcinogens. Most saponins aglycones consist of either pentacyclic triterpenoids or tetracyclic steroids. They show immune modulating and antineoplastic effects. Anthraquinone glycosides are relatively limited distribution within the plant kingdom. Their primary effect is induction of water and electrolyte secretion as well as peristalisis in colon.[14]

4.3. Total phenols concentration in the methanolic extract of *Coriander sativum* leaves

From the Figure – 2, 3, 4, and Table – 2, 3 the total phenolic content determined by the Folin-Ciocalteau reagent will not exhibit the accurate quantity of the phenolic composition in the extracts and hence HPLC determination of polyphenols was performed.[15] The amount of polyphenols present in *Coriander sativum* leaves extract is 320 µg /ml. There are about 21 phenolic compounds were identified in the part of coriander leaves. These are mainly flavonoids, coumarins, and phenolcarboxylic acids. These compounds, includes apigenin, luteolin, hyperoside,hesperidin, vicenin, diosmin, orientine, dihydroquercetin,chrysoeriol, catechin, ferulic acid, gallic acid, salicylic acid,dicoumarin, 4-hydroxycoumarin, esculin, esculetin, maleic acid, tartaric acid, and arbutin.[16] The polyphenol composition includes dimethoxycinnamoyl hexoside, quercetin-3-O-rutinoside, quercetin 3- O-glucuronide, quercetin-3-O-glucoside and kaempferol-3-O-rutinoside as the main phenolic acids. Along with phenols and polyphenols flavonol derivatives including 3-O-caffeoylquinic acid, caffeoylquinic acid, ferulic acid glucoside and p-coumaroylquinic acid are the four flavonoids identified in coriander leaves.[17] The major phenolic acids identified from India are vanillic, p-coumaric, cisferulic and trans-ferulic acids and the flavonoids are kaempferol, quercetin, 3′-OMe quercetin, 4′-OMe quercetin and acacetin. And hence in Indian coriander leaves found to contain quercetin.[18]

4.4. Antioxidant property in the methanolic extract of *Coriander sativum* leaves

From the Figure – 4 FRAP assay and reduced glutathione estimation measures the reducing ability of antioxidants against oxidative effects of reactive oxygen species. Total antioxidant power may be referred analogously to total reducing power. The total amount of antioxidants present in the methanolic extract of *Coriander sativum* leaves by FRAP assay was found to be 480 µg / ml and by estimation of reduced glutathione was found to be 40 µg / ml. Antioxidant activities are mainly attributed to the active compounds present in them. This can be due to the high percentage of main constituents such as polyphenols, flavonoids, terpene, terpenoids etc. as well as to the presence of other constituents in small quantities or to synergy among them.[19] The polyphenols present in *Coriander sativum* leaves extract are about 21 different compounds. Terpene and terpenoid components such as camphor, limonene, α-pinene and geraniol also contribute the total antioxidant activity.[20]

5. CONCLUSION

The phytochemicals present in the methanolic extract of *Coriander sativum* leaves contains Tannins / Phenols, Terpenoids, Carbohydrates, Amino Acids and Proteins. Overall these phytochemicals possess various medicinal activities. The total amount of carbohydrates and proteins present in the methanolic extract of *Coriander sativum* leaves was found to be 37 µg/ml and 120 µg/ml which are mostly in the form of glycosides. The amount of Polyphenols present in *Coriander sativum* leaves extract is 320 µg/ml. The major phenolic acids identified from India are vanillic, p-coumaric, cisferulic and trans-ferulic acids and the flavonoids are kaempferol, quercetin, 3′-OMe quercetin, 4′-OMe quercetin and acacetin. The total amount of antioxidants present in the methanolic extract of *Coriander sativum* leaves was found to be 480 µg / ml which may be due to the presence of number of polyphenolics such as flavonoids, anthocyanins etc.

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