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Isolation and determination of some sugars of *allium cepa* linn.(liliaceae) using column chromatography

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SUMMARY

The sugars in *Allium Cepa*Linn.(onion);was isolated and fractionated using silica gel filtration technique.Thespectrum absorption of the isolated fractions by silica gel column was measured at 620 nm after treating each isolated fraction with anthrone reagent. The molecular weight of these fractions was calculated and found to be ranging: 530,190,140 Daltons.The paper chromatography showed 5 spots three of which have been detected as glucuronicacid ,galactose and mannose. In conclusion the information derived may be useful in the determination of *Allium cepa*Linn.(onion)'s contents in Iraq.

Key words: Allium Cepa L.; column chromatoghyaphy method, sugars.

1.Introduction

*Allium cepa*Linn.(onion)is one of the world's most widely cultivated vegetables, with their culinary and medicinal uses spanning history and the globe. *Allium cepa*Linn.(onion)'s health benefits, for it contains a range of phytochemicals with an array of biological effects, including antioxidant activity. There is an evidence that *Allium cepa* Linn play an important role in protecting against major chronic diseases as well as health problems associated with ageing.

Its antimicrobial activity, long recognized in folk remedies, has also now been scientifically validated. *Allium cepa*L.is usually thought of as a vegetable, it also has a long history of medicinal use.

Mainly the fleshy bulb that grows below the ground is used medicinally as well as for food but

other parts of the plant also have a place in the traditional medicines. *Allium cepa*L.(onion) bulbs are white, yellow, or red. The green stems and leaves are hollow [1-3].

Botanical Source

The common onion is a garden plant, having a scape, which appears the second year, 2

to 4 feet high, being naked, smooth, straight, stout, swollen at the base, and fistulous, bearing at

the top a round umbel of greenish-white flowers. The leaves are round and fistulous, of a shining
green color, acute, and shorter than the stem. The part employed is the bulb.

Description

The *Allium cepa*L.(onion) is a bulb, compressed or round, or oblong in figure, invested with a shining ,thin, dry membrane, of a reddish or white color. It is less pungent to the taste than garlic, withsome degree of sweetness, and a peculiar, well-known odor. Onion bulbs are of various shapesand sizes, usually globular, the layers being juicy.

Scientific classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Liliopsida
Order: Asparagales
Family: Alliaceae
Genus: *Allium*
Species: *A. cepa*
Edible Parts: Flowers, Leaves, Root, Seed.

Nutrition value of onion

Values per 100 gms edible portion
Moisture 86.6% , Calcium 47 mg
Protein 1.2% ,Phosphorus 50 mg
Fat 0.1% ,Iron 0.7 mg
Minerals 0.6%, Vitamin C 11 mg
Carbohydrates 11.1% ,Fibre 0.4% [3]

*Allium cepa*L. is a member of the Liliaceae,which consists of over 250 genera and 3700species. Because of their bulbs, tubers and rhizomes, these plants are able to survive under harsh conditions, e.g. winter or dryness. The plant *Allium cepa*L. (Liliaceae) are proved to shown the antidiabetic ,antioxidant, antihypertensive, anti
thrombotic,hypoglycemic,antihyperlipidemi c .

The structure and mechanical properties of onions are important factors affecting their textural quality. The onion bulb consists of several layers of pigmented, papery scales surrounding fleshy storage scales that comprise an upper epidermis, an intermediate parenchyma tissue, and a lower epidermis.

*Allium cepa*L. (onions) are rich in phenolic compounds. Moreover, they are a major source of quercetin, a flavonol used as anutritional supplement for its anti-inflammatory and antioxidant properties.

Onion extract shows antioxidants properties and may be beneficial against some cancers. Indeed, onion extract inhibited cell proliferation of colon, kidney and liver cancer and could inhibited mutation processes that triggers cancer. Onions also have hypoglycemic properties and the capacity to decrease platelet aggregation. Otherin vitro studies have shown that onion juice or extract have anti-parasitic, anti-fungal and anti-bacterial properties. Recent researches have reported that phenolic phytochemicals from onion have blood glucose lowering effect and high antioxidant activity in alloxan-induced diabetic rat**.**

Total antioxidant capacity by Phosphomolybdenum method assay is based on the reduction of
Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo
(V) complex at acidic pH. The total antioxidant capacity of methanol extracts of *Allium cepa*L. is *(*29μg).

In Indian folk medicine, the bulb of *Allium cepa*L.is used to treat dysentery, fever, chronic bronchitis, insect bites, stings, skin diseases . The anthelmentic activity has a linear relationship with the dose of the extract used; the earthworms were paralysed within 20 mins and died within 30 mins of exposure. [4-10]

The plant *Allium cepa*Linn. (Liliaceae) are proved to show the antidiabetic, antioxidant, antihypertensive, antithrombotic,
hypoglycemic,antihyperlipidemic.

Bulb extract shown to have ecobolic effect in rats. The bulb of of*Allium cepa*L.

containsKampferol, _sitosterol, ferulic acid, myritic acid, prostaglandins.Traditionally plant containing these constituents used as abortifaciant, the bulbextract of *Allium cepa*L.had shown ecobolic effect in mice and rats[11].

The use of plant extracts and phyto-chemicals, both with known antimicrobial properties, have been identified by the World Health Organization as alternative sources of therapeutic treatments (World Health Organization - WHO, 2001) . The beneficial medicinal effects of plant materials typically result from the combinations of their secondary metabolites; and their medicinal actions are unique to particular plant species or groups and this is based on their distinct taxonomy. Plant-derived drugs thus serve as a prototype to develop more effective and less toxic medicines [12].

The nutritional composition of onion is very complex. It has been shown that it is one of the major sources of dietary flavonoids in many countries. Specifically, onion has been characterized for its flavonolquercetin and quercetinderivates. Moreover, it is rich in other bioactive compounds such as fructooligosaccharides and sulphur compounds. Among the organosulphur compounds identified in onion oil and powder is dipropyldisulfide, one of the major isolated chemicals [13]

The aim of the present study was to extract, separate and determine the carbohydrates' contents of *Allium cepa*Linn using column chromatography which permits a rapid and effective separation technique.

2.MATERIALS AND METHODS:

Preparation of*Allium cepa* Linn extract:

The *Allium cepa*L extract was prepared using healthy dry yellow bulbs of onions. The onion used in this experiment was obtained from the local market in Basra city south of Iraq. 200g of onion were collected, weighed and washed under running tap water and with distilled water to remove surface impurities. The*Allium cepa*L. (onion) was minced using a mixer grinder. After homogenization, crushed onion was filtered. The filtrate was placed in incubator in order to be concentrated. The concentrated *Allium cepa*L was dissolved in distilled water and used for the qualitative analyses to know the presence of various biochemical contents in the extract [14-15].

Qualitative Analysis:

Ultraviolet and Visible Spectroscopy:

A diluted *Allium cepa*L extract was used to measure the absorption spectra against a distilled water as a blank using spectrophotometer[UV-1100 EMS LAB spectrophotometer]. The measurements are made in the range {200-640} nm, the result on (fig: 1).

Detection of carbohydrates:

Molish test: To 1 ml of the diluted test solution (*Allium cepa*L. extract), two drops of Molish reagent (5% naphtol in alcohol) were added, shaken well, 1ml of conc. sulphuric acid was added slowly along the sides of the test tube, violet ring indicated the presence of carbohydrates.

Barfoed's test: To 1ml of diluted test solution, 1ml of Barfoed's reagent (6.5% cupric acetate in distilled water, filtered and added 0.9 ml of glacial acetic acid) was added and heated in a boiling water bath for 2min. Red precipitate indicated the presence of reducing monosaccharides.

Benedict's test: To 0.5ml of diluted test solution, 0.5ml of Benedict's reagent (173g sodium citrate and100g sodium carbonate

was dissolved in 800 ml of distilled water and boiled to make it clear(17.3g cupric sulphate dissolved in 100 ml distilled water was added to it) was added
and heated in a boiling water bath for 2 min. Red precipitate indicated the presence of reducing sugars[16].

Detection of amino acids and proteins: The *Allium cepa*L. Extract was subjected to qualitative tests for amino acids.

Biuret'sTest: To 1 ml of diluted test solution,3 drops of sodium sulphate (0,5 gm of sodium sulphate in 100 ml distilled water) were added, shaken well, 1ml of sodium hydroxide (10%) was added, shaken well. Purple color indicated the presence of proteins and peptides.

Ninhydrin's Test: To 1ml of diluted test solution, 1 ml of ninhydrin reagent (0.2 gmof ninhydrin in 100 ml ethanol) added,and heated in a boiling water bath for 2 min. Blue-purple color indicated the presence of amino acids, the result on table:1[17].

Pauly's Test: To 1ml of test solution, 1ml of sulphanilic acid was added followed by 1ml
of 5% sodium nitrate solution. After 3min, solution was made alkaline by adding 2ml of 1%Na2Co3 solution. The appearance of yellow, orange and red colour indicated the presence of
tyrosine, tryptophan and histidine respectively.

Xanthoproteic's Test: To 1ml of test solution, 10 drops of concentrated nitric acid was addedThe appearance of yellow color indicated the presence of phenol containing amino acids like tryptophan and tyrosine, the result on (table :1).

Paper Chromatography:

*Allium cepa*L. Extractapplied to the WhatmanNo.1 paper strip(10x15)cm with analytical gradeof sugarscontaining galactose,mannose,raffinose ,lactose and arabinose. Then the paper dipped in the solvent system containing [n-butanol:toluene:pyridine:water(5:1:3:3 v/v)].The paper allow to dry in order to be developed by (aniline hydrogen phthalate AHP)(9.2 ml of aniline dissolved with 16 gm.phthalic acid in n-butanol(490ml),ethanol(490ml)and distilled water (20ml) ,[14]the result on (table :2).

Column Chromatography of *Allium cepa* L.Ex**tract:**

A column of silica gel (12x1 cm) was employed after being washed with distilled water. Thecolumn was eluted with distilled water and at a flow rate of 2 ml per 15 min .The column was calibrated with analytical gradeof
sugars:(Raffinose,540;Lactose,360;Mannose,180 and Arabinose,150)Dalton. A molecular weight calibrated curve (Fig.2) defines the relationship between the elution volumes of a set of these sugars and their respective mol.wt.

*Allium cepa*L. extract (1.0 ml) was applied to the column. Step-wise elution was made with three kinds of eluent systems of varying ionic strength: distilled water; 1.5 % ethanol and 15%of ethanol, the eluent was collected 2ml/15min. Each fraction treated with Anthronereagent (0.2 gmAnthrone dissolved in 100 ml of concentrated sulphuric acid).An ultra-violet absorption measurements of each fractionat620nrn using spectrophotometer was made, values of absorption were plotted against the elution volume (Fig. 3), then the molecular weight of the separated fractions was calculated [14-15].

3.RESULTS and DISCUSSION:

The UV and Visible spectra of *Allium cepa*L. extract (fig:1) shows big peak in the range (220-280) nm which indicates the presence of nucleic acids , peptides and proteins. Another peak appeared at absorbance 420 nm which indicates the presence of yellow Anthraquinone pigments and aromatic amino acids .Other peaks appeared at absorbance 560 nm and 640nm indicated to the presence carbohydrates and amino acids[14].

The results of carbohydrates , amino acids and proteins screening in terms of qualitative tests carried out to identify carbohydrates, amino acids and proteins in *Allium cepa*L. extract are presented in (Table :1).

Table1. Carbohydrates, amino acids and proteins screening in*Allium cepa* L. extract

Phytochemical Constituents	**The extract**
Carbohydrates	
a. Molish test	+
b. Barfoed's test	+
c. Bendict's test	+
Amino acids and proteins	
a. Ninhydrine's test	+
b .Xanthoproteic's test	+
d. Pauly's test	+
e. Biurat's test	-

In the present investigation, various tests (Molisch, Benedict's, Barfoed's) carried out for carbohydrates for *Allium cepa*L. extract showed positive response for carbohydrate, reducing sugars as well as reducing monosaccharides.

Carbohydrates obtained from natural sources such as plants have shown diverse biological activities such as wound healing, enhancement of the reticulo endothelial system, stimulation of the immune system, treatment of tumours and effects on the hematopoietic system [4,18].

In the present study, Millions test indicative of the presence of phenol containing amino acids (tyrosine), Xanthoproteic's test, positive for phenol containing amino acids like tryptophan and tyrosine; Pauly's test positive for imidazole containing amino acid like histidine.

The results for paper Chromatographyfor *Allium cepa*L. extract using WhatmanNo.1 paper strips and developed by Aniline hydrogen phthalate solvent system; shows the presence of glucuronic acid(Rf:3) ,galactose (Rf:19) and mannose (Rf:30) .Also it shows two unknown spots with Rf : 7 and 10 respectively (Table:2).

Table:2 The carbohydrates contents in *Allium cepa* L. extract and their Rf with it's Rf values of standard sugars.

Spot No.	Sugar	Rf(*100) Result	Rf(*100)STD
1	glucuronic acid	3	3
2	unknown	7	-
3	unknown	10	-
4	galactose	19	21
5	mannose	30	29

The elution curve of sugar fractions during the three kinds of buffer systems of varying ionic strength at 620nrn after treated with Anthrone reagent is shown in Fig.3, With distilled water as a first eluent, one big peak appeared in the curve at an absorbance value (1.94). While during the elution with 1.5% ethanol as a second eluent, one peak appeared at absorbance value (0.42). When we used 15%Ethanol as a third eluent; one peak appeared with absorbance value (0.33). The fractions corresponding to these peaks were named as F_I. F_{II}, F_{III} respectively.

The molecular weight of the isolated fractions was calculated by using the best fit line from the standard curve (fig:2)and applied the value of the volume that corresponds to each appeared peak in (fig:3). The molecular weight of the first fraction (F_I) was530 daltons, the second fraction (F_{II}) was 190daltons and the third fraction (F_{III}) was 140 daltons after compared with the standard curve (fig:2).The isolated fractions were less or more in the number of Mwt when compared with the standard sugars above;this may be due to the differences or manners of procedure used in both elution steps and spectra measurements for both the standard and the extract.

The gel filtration chromatography seems to be an adequate method for sugar and protein separation because it separates molecules according to size: the large molecules are excluded and emerge in the void volume, and the small molecules which can enter, the smaller molecules are retarded. So, this analytical method may be useful method to separate and purified molecules such as carbohydrates and proteins [18].

ln conclusion, this study is considered as a part of series studies accomplished on the *Allium cepa*L. extract and these results are evidence of qulititative differences of sugars and other contents in *Allium cepa*L.may due to genetic ,quality and storability factors.The gained data of the present study added an interesting information for further studies in *Allium cepa*L. from local market in Basrah city in Iraq.

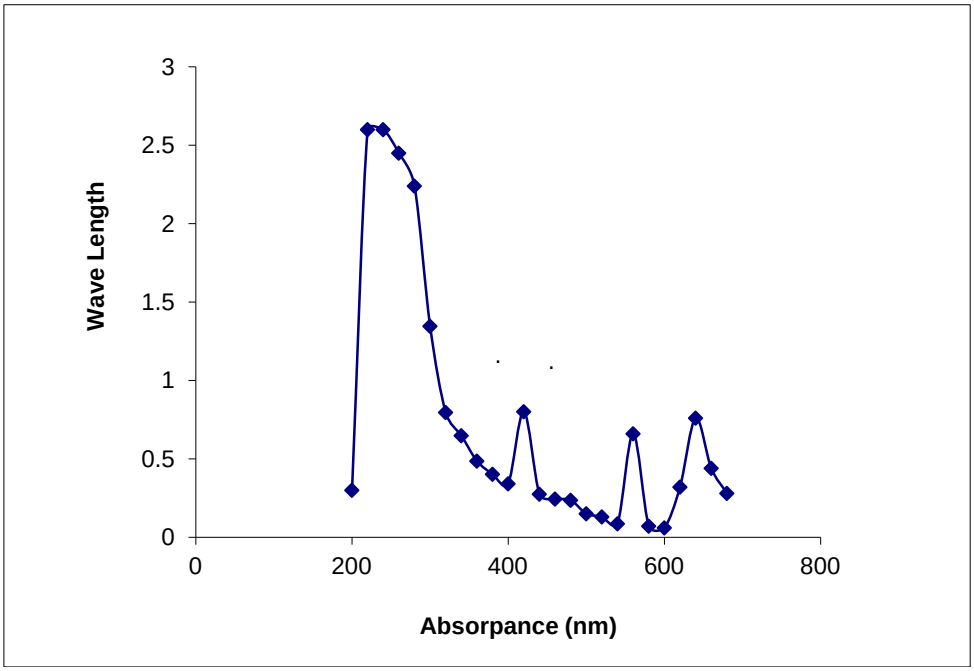

Figure.1. UV and Visible Absorption Spectrum of *Allium cepa*L. extract.

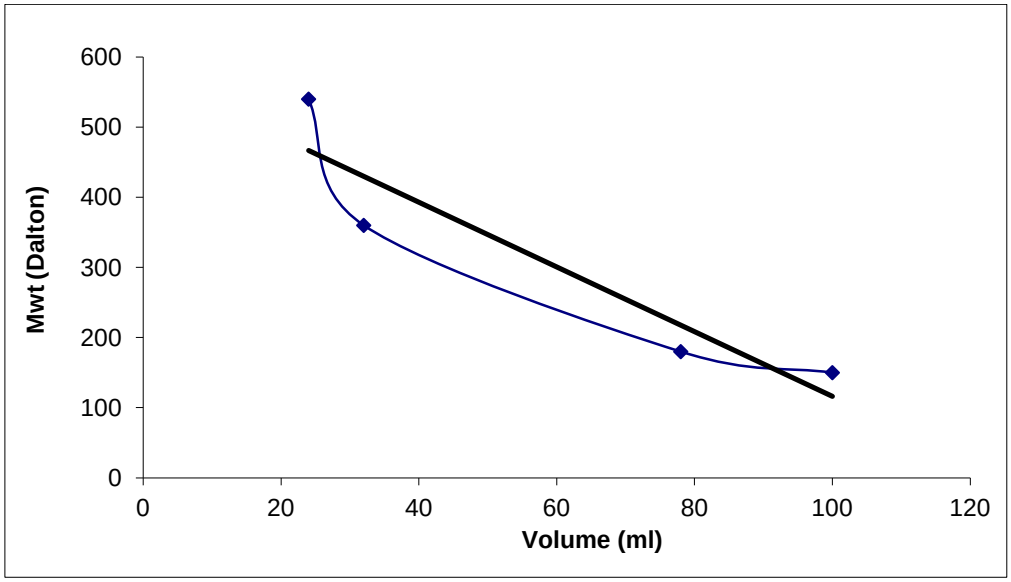

Figure.2.A standard curve to estimate sugar's molecular weight using correlation between Mwt(Dalton) vs elution volume(ml) of the standard sugars.

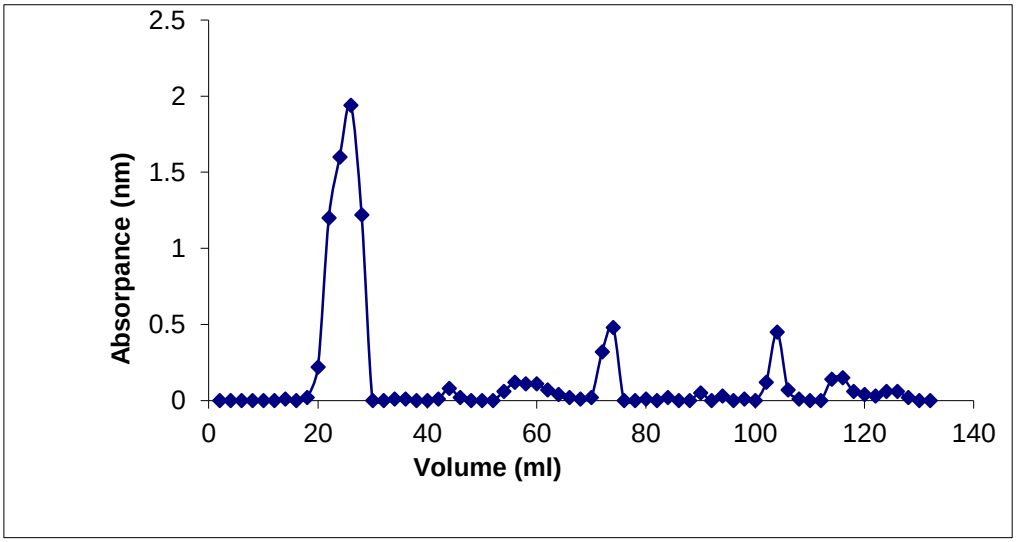

Figure.3. Elution curve of *Allium cepa*L. extract using silica gel column Chromatography measured at absorbance 620 nm.

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عزل وتشخيص السكر المفصول من مستخلص(Liliaceae).*ALLIUM CEPA* LINN وتشخيصه باستخدام كروموتوغرافيا الترشيح الهلامي تقنية

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الخلاصة:

تم عزل وتشخيص السكريات المفصولة من مستخلص *Allium cepa*L. وباستخدام هلا م السليكا في تقنية كروموتوغرافيا الترشيح الهلامي ,وقيس الامتصاص الضوئي للأجزاء المفصولة عند الطول الموجي (620) نانوميتر بعد معاملتها مع كاشف الا نثرون ,وقيست الاوزان الجزيئية لهذه الأجزاء ووجد أنها تتراوح بين140,190,530دالتون, كما اظهرت كروموتوغرافيا الورقة خمس بقع بعد تظهيرها, ثلاث منها تشير الى وجودالسكريات وبعض الاحماض السكرية مثل الكالاكتوزوالمانوزوحامض الكلوكورنك . وقد قدمت النتائج الحالية بان التقنية المستخدمة مفيدة في معرفة مكونات *Allium cepa*L. من النوع الموجود في البصرة جنوب العراق.

الكلمات المفتاحية: *Allium cepa*L. , طريقة عمود الكروموتوغرافيا,سكريات.