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IDENTIFICATION AND ESTIMATION OF CARBOHYDRATE AND AMINO ACIDS CONTENTS OF MUCILAGE FROM CALLUS CULTURES OF *PLANTAGO LANCEOLATA* L.

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Abstract

Mucilage is a natural metabolic bi-product of *Plantago lanceolata* L. was isolated from seeds, plant leaves and callus cultures. Callus was derived from the midrib of the leaf of 21 days old sterile seedlings of *P. lanceolate*. Results obtained using High Performance Liquid Chromatography (HPLC) was used to identify the isolated mucilage content and estimate the percentage of the individual components of both carbohydrate and amino acids.

Galactose was found to be the major sugar present with 902.75 µg/ ml (36.15%) in dry callus. The highest percentages of amino acids were recorded by both aspartic acid and glutamic acid {(1142.24 and 1026.23 µg/ ml), (9.88 and 8.88%)} respectively in air-dried callus. Mucilage content was also measured in dry leaves, it was found that the dry callus contained three times of mucilage higher than the dry leaves. These findings indicate that callus cultures are the most favorable and continuous source of plant mucilage.

Keywords : Mucilage, *Plantago lanceolata*, Callus induction, tissue culture.

Introduction

Plantago belongs to the family *Plantaginaceae*. The species *Plantago lanceolata* is a widely distributed wild plant (Tack and Liisa-laine, 2014). Its seeds are distributed for long distances, due to their attachment to the feet of any person passing over as they become sticky when they are wet because their pericarps contain mucilage (Kreitschitz *et al.*, 2016). It is an annual plant grows in the summer and has a long history in treating many diseases such as bronchitis, whooping cough, tuberculosis, indigestion, gastrointestinal disturbances, cystitis, nocturia, burning urine, diarrhea, and also used to strength the bodies of weak children (Al-Maadhidi, 2007). The therapeutic properties of the plant are related to its contents of mucilage, glycosides, especially aucubin and flavonoids (Abdul Ratha and Mohammad, 2012).

Plants are important source of natural products, a large number of these products constitutes the basic constituent of many drugs that have medical importance despite the recent advances in the drug industry (Jain *et al.*, 2012). Plant mucilage is a natural metabolic product formed intracellularly and precipitated as layers that help the plants to store water and in seeds germination. In addition mucilage serves as a membrane thickener and as food reserve for the plant (Bhardwaj *et al.*, 2000). This metabolite is found in different parts of the plant for example, the roots, phloem, seed cortex and leaf epidermal cells (Vishwakarma *et al.*, 2004).

Tissue culture technology and in vitro regeneration of cells and organs are the most important methods which provide advantages of a large productivity regarding the quantity and the quality, and controlled production away from the restriction of the natural environmental factors, such as the geographical location and seasonal changes or environmental stresses (Zhou and Wu, 2006). The need for the numerous number of medical, pharmaceutical and dyes materials, increased recently (Mulabagal and Tasy, 2004). This need requires the use of tissue culture technology, all of a sudden plant cell and tissue culture, became the fastest typical source, for the in vitro production of continuous large quantities of pharmaceutical compounds, away from the constraints of the environmental conditions, without its interference with other compounds, as might happen during its isolation from the whole plant (Karuppusamy, 2009).

Golkar *et al.* (2017) made an attempt to improve the mucilage content in 14 different types of hereditary patterns of *P. ovata* Forsk, using the terminal buds, cotyledonous leaves and previously laboratory germinated seeds. They found that the callus mucilage content was three folds higher than that of the seeds.

This project aimed to estimate the callus mucilage content and to determine its components of carbohydrate and amino acids.

Materials and Methods

Seeds were sterilised, cultured and grown until the age of seedlings became 21 days to be used in the experiment, according to the method described by Al-Mahdawi (2013). Then the bases of the leaves from intact sterile seedlings of 1.0-1.5 cm, were separated and explants were taken, from the midrib of the leaves and transferred for callus induction on selected Murashige and Skoog (MS) medium supplemented with 0.5, 1.0, 1.5 and 2.0 mg/ l 2,4-Dichloro phenoxy Acetic Acid (2,4-D) combined with 0.5 mg/ l Kinetin (Kin). One segment was used per each 250 ml flask containing 20 ml of the medium. Then incubated in growth room at 25 ± 2°C for a consecutive photoperiod (16 h light/ 8 h dark), (Al-Mahdawi, 2013).

Isolation of the crude mucilage from the seeds, the air-dried leaves and the air-dried callus of *Plantago lanceolata* L. were performed. One gram from each was taken and crude mucilage was isolated according to methods described by other workers (Sharma and Koul, 1986; Kardosova, 1992).

Then the crude polysaccharides were isolated from the mucilage of the seeds, air-dried leaves and air-dried callus, according to method previously described by Kardosova (1992). Identification of carbohydrate components were performed by apparatus of high performance liquid chromatography (HPLC) Shimaduz 10A V-LC supplied with a conduction pump type Shimaduz LC-10A. The data of the area under the curve and those of the retention times of the samples, were compared with that of the standard sample by which the crude mucilage`s carbohydrate can be identified. Carbohydrate concentrations are calculated according to the following equation:

$$(X)\text{Concentration} = \frac{\text{Area of the sample`s band}}{\text{Area of the standard`s band}} \times$$

The standard concentration × Number of dilutions

Amino acids were isolated and identified from the crude mucilage of seeds, air-dried leaves and air-dried callus by method described by Fierabracci *et al.* (1991). Using HPLC technique (Furst *et al.*, 1990). The used HPLC apparatus was supplied with two pumps type LC-6A (Koyoto, Japan), also possess SIL-6A automatic apparatus together with another apparatus of UV detector type Shimadzu SPd-6AV UV, which has 8 micron a flow cell. The amino acids were identified and estimated by comparing their areas under the curves and their retention times with those of the standard sample.

Results and Discussion

Table (1) shows the mucilage contents from seeds, dry leaves and dry callus. The highest mucilage content was obtained from callus 14.34 mg for the wet mucilage and 0.03 mg for dry mucilage. Seeds gave 12.77 mg of wet mucilage which became 0.02 mg when dried. However, air-dried leaves gave the lowest amount of wet mucilage (7.15 mg) which was equal to 0.01 mg dry mucilage. It is clear from the above results that the callus is the best source of mucilage which was 3 times higher than that of dry leaves. These results agreed with those reported by Mirmasumi *et al.* (2001) who mentioned that the callus is the best source of mucilage compared with other plant organs.

Although there have been other studies that analysed the mucilage contents of carbohydrate and amino acids (Karodova, 1992). However, none so far has performed such analysis on the mucilage obtained from callus.

Carbohydrate were separated from mucilage contents from seeds, air-dried leaves and air-dried callus of *P. lanceolata* L. according to method previously used by Kardosova, (1992). The recorded areas under the curves deduced from high performance liquid chromatography (HPLC) technique (Fig. 2) were used to estimate the carbohydrate contents and their individual components in the mucilage. These results showed that the main sugar component was D-galactose in all callus, leaves and seeds (36.15%, 35.54% and 30.85%) respectively (Table 2). The second major components in callus was D-galacturonic acid, which constitutes 25.13%. Arabinose stood to be the second main component in seeds (24.95%) and the leaves (22.0 %). These differences could be due to the organ variations.

It was observed that the total carbohydrate concentration in dry callus was (2658.36 µg/ mole). This value was higher than that of dry leaves (2326.12 µg/ mole) and that of the seeds (2263.39 µg/ mole). In general, our results agree with the results reported by Kardosova (1992) with respect to the sugar components of Carbohydrate isolated from the crude mucilage. However, there are slight differences in the percentage of some sugar components, for example, Kardosova (1992) reported a percentage of arabinose (26%) in leaves which is slightly higher than the percentage we found in the leaves (22%), this could be due to geographical variations.

Amino acids HPLC profiles highlighted in (Fig. 3) showed the amino acid composition of the crude mucilage from the seeds, dried leaves and dry callus of *P. lanceolata*. The results revealed that Aspartic acid from the dried callus has had the highest value which was 1142.24 µg/ ml (9.88%) (Table 3). The second highest value of amino acid from callus was recorded by Glutamic acid which was 1026.23 µg/ ml (8.88%). Alanine and proline were slightly different from glutamic acid with percentages reached 8.42% and 8.05% respectively. However, glutamine had the least value which was 94.34 µg/ ml (0.81%). No glutamine was detected in the seeds sample. Total concentration of amino acids contents in seeds in terms of µg/ ml, seeds seems to be having the lowest concentration compared with dry callus and dried leaves.

Among the amino acids recorded in callus, there was no tryptophan in the callus samples. The picture with respect to amino acid analysis of the air-dried leaves samples was slightly different, the highest concentration was recorded by glutamic acid which was 1035.64 µg/ ml (9.26%). The second highest concentration was of alanine which reached 973.36 µg/ ml (8.7%). Aspartic acid had the third highest concentration of 950.33 µg/ ml (8.50%). Tryptophan appeared at low concentration of 110.94 µg/ ml (0.99%). Glutamine showed the lowest concentration (0.85%). Table (3) show differences in the percentages of individual amino acid among seeds, dry leaves and dried callus samples. These differences could be attributed to orang/ tissue specificity.

Our results of air-dried leaves agreed with those obtained by Kardosova (1992), in that the highest percentage of amino acid recorded was by glutamic acid. However, aspartic acid was the third highest concentration in our study. Comparing our results of amino acid composition of mucilage from seeds, air-dried leaves and air-dried callus, we can find that aspartic acid had the highest percentage among amino acids in dry callus samples. Whilst glutamic acid has had the highest percentage in both the seeds and the dried leaves, recording the highest percentage in the seeds sample (10.65%). These differences between the three sets of results can be explained again on the basis of tissue variations.

Kardosova *et al.* (1989) worked on the mucilage from the leaves of the marsh mallow (*Althaca officinalis* L., var Rhobusta), also showed that aspartic acid, glutamic acid and alanine were among the highest accumulated amino acids. However, they showed that glycine percentage (10.56%) was almost twice the percentage we recorded in our study (6.25%). This could be explained due to the species differences.

In conclusion callus seems to be the best source of mucilage from *Plantago lanceolata*. The quality of mucilage obtained from callus is judged by its components of carbohydrate and amino acids.

Table 1 : Mucilage contents of seeds, air-dried leaves and air-dried callus from the plant *Plantago lanceolata* L.

The sample source	Wet mucilage	Dry mucilage
Seeds	12.77 mg/g	0.02 mg/g
Leaves	7.15 mg/g	0.01 mg/g
Callus	14.34 mg/g	0.03 mg/g

Table 2 : Components of carbohydrate isolated from mucilage of *Plantago lanceolata* L. using HPLC.

Carbohydrate		Seeds		Dry leaves		Dry callus
Concentration µg / ml	Percentage %	Concentration µg / ml	Percentage %	Concentration µg / ml	Percentage %	
D-galacturonic acid	470.09	20.76	489.62	21.04	668.12	25.13
D-galactose	698.27	30.85	826.84	35.54	902.75	36.15
Mannose	252.31	11.14	225.96	9.71	325.95	12.26
Rhamnose	277.55	12.26	271.92	11.68	462.17	17.38
Arabinose	565.17	24.97	511.78	22.00	299.37	11.26
Total	2263.39		2326.12		2658.36	

Table 3 : Amino acid composition of crude mucilage from the seeds, air-dried leaves and air-dried callus of *Plantago lanceolata* L. using HPLC.

Amino acids	seeds		Dry leaves		Dry callus	
	Concentration µg / ml	Percentage %	Concentration µg / ml	Percentage %	Concentration µg / ml	Percentage %
Aspartic acid (Asp)	462.53	7.08	950.33	8.50	1142.24	9.88
Glutamic acid (Glu)	695.38	10.65	1035.64	9.26	1026.23	8.88
Glutamine (gln)	0	0	95.47	0.85	94.34	0.81
Glycine (Gly)	408.35	6.25	664.07	5.94	635.35	5.49
Histadine (His)	226.23	3.46	285.09	2.55	360.24	3.11
Arginine (Arg)	287.08	4.39	673.91	6.03	530.70	4.59
Theronine (Thr)	493.97	7.56	801.00	7.16	690.72	5.97
Alanine (Ala)	456.24	6.98	973.36	8.71	973.49	8.42
Proline (Pro)	728.35	6.13	798.21	7.14	929.97	8.05
Tyrosine (Tyr)	491.75	7.53	713.75	6.38	616.44	5.33
Valine (Val)	264.98	4.05	870.42	7.78	622.81	5.39
Methionine (Met)	164.03	2.51	418.36	3.74	373.76	3.23
Cystine (Cys)	362.70	5.55	763.34	6.33	964.84	8.35
Tryptophan (Trp)	0	0	110.94	0.99	0	0
Isoleucine (Ile)	168.59	2.58	357.61	3.20	416.63	3.60
Leucine (Leu)	388.69	5.95	656.74	5.87	794.45	6.87
Phenylalanine (Phe)	412.16	6.31	377.94	3.38	498.59	4.31
Lysine (Lys)	516.90	7.91	628.28	7.41	881.56	7.63
Total	6527.93		11174.46		11552.36	

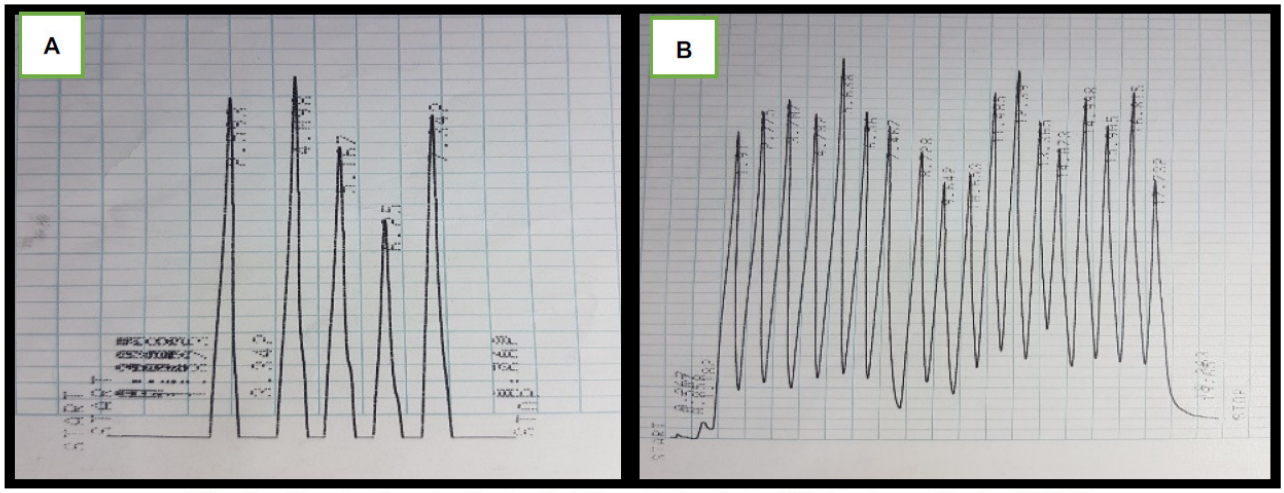

Fig. 1 : Standard peaks for both (A) carbohydrate (B) Amino acids by HPLC.

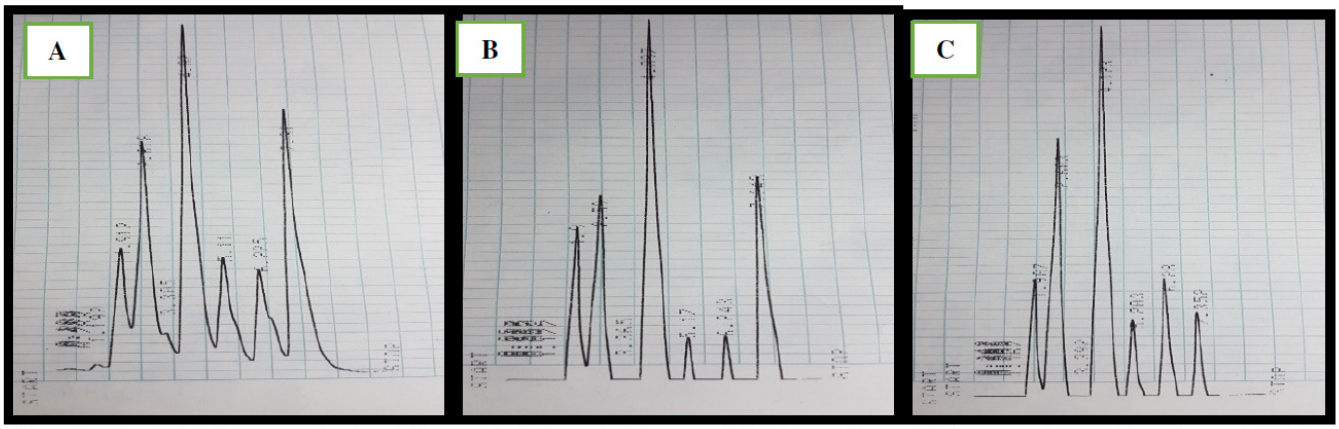

Fig. 2 : Carbohydrate peaks for (A): seeds (B): dried leaves (C): dry callus of *Plantago lanceolata* L. by HPLC.

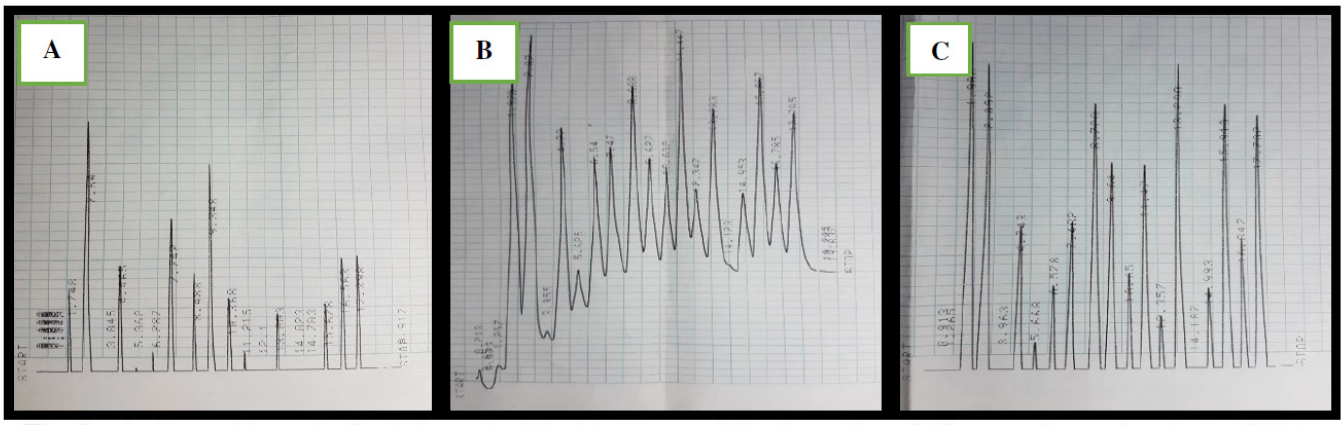

Fig. 3 : Amino acids peaks for (A): seeds (B): dried leaves (C): dry callus of *Plantago lanceolata* L. by HPLC.

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