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ANATOMICAL AND CHEMICAL INVESTIGATIONS ON *ASPARAGUS OFFICINALIS* L. (ASPARAGACEAE)

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ABSTRACT

Although Asparagus (*Asparagus officinalis* L.) is chiefly known as a vegetable herb, little information about the botanical characteristics of such plant are available. Therefore, it is aimed in this study to bring light more information about the morphological, anatomical and some important chemical contents of vegetative and reproductive organs of the plant, throughout the consecutive stages of its entire life span under the local conditions. Seeds of Asparagus (cv. Mary Washington 500 W) were sown 2nd Feb. 2013. The field experiment was carried out at the Experimental and Research Station of Fac. of Agric., Cairo Univ., Giza, Egypt during the two successive growing seasons 2013 and 2014. Asparagus is a herbaceous, perennial plant that grows up to 1.5- 2 m height. The plant posses aerial stems (ferns) and much-branched feathery foliage. The leaves are triangular scales like, 3-7 mm in length. The cladodes (modified stems) arise in the axis of scale leaves . Cladodes are found in fascicles (3-6) on each node. The flowers are bell- shaped, greenish – white to yellowish, 4.5-6.5mm long, with 6- tepals,single or in clusters of 2-3. Anatomical studies were carried out for various organs of Asparagus plant including, apex of the aerial stem, visible internode below shoot apex, median portion of the aerial stem, the cladode , median portion of spear, scale - like leaf, rhizome, adventitious root. The major chemical metabolites contents of spear were determined.

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INTRODUCTION

Asparagus is a large genus with over 160 different species of herbaceous perennials crop of high economic value. The most economically important asparagus (*Asparagus officinalis* L.), which is a highly prized dioeciously nontraditional vegetable crop **(Stajner et al 2002)**. Tender and unexpanded shoots, commonly called spears, are the edible organs of garden asparagus and a planting can produce spears for up to 15 years **(Rubatzky and Yamaguchi, 1997)**. Other species used mainly as ornamental or medicinal plants. Asparagus is one of the most nutritionally well balanced vegetables in existence, which is high in folic acid, thiamin, vitamin B6 and a good source of potassium.

Modern taxonomists have placed the genus *Asparagus* in family Asparagaceae of Order Asparagales rather than in Liliaceae, then, the family Asparagaceae contains two other genera, *Asparagopsis* and *Myrsiphyllum*, and about 370 species, most of them are cultivated as ornamental or medicinal plants **(Ali and Khan, 2009). Fellingham and Meyer (1995)** determined that the family Asparagaceae is recognized by having rhizomes, branched aerial stem ,scale– like leaf, cladodes ,(modified stems) and axillary flowers or axillary inflorescences.

Although *Asparagus officinalis* L. is chiefly known as a vegetable herb, little information about the botanical characteristics of such plant is available. Therefore, the objectives of this study was to investigate the anatomical structures of different vegetative organs during different developmental stages of plant life span. Moreover, major metabo-

lites analysis and chlorophyll content of the plant was carried out. This would be an effort to proper delimitation of this species of the family Asparagaceae and even more. Such knowledge would be useful to specialists in various aspects of biology of such important economic plant.

MATERIALS AND METHODS

The current investigation was performed on *Asparagus officinalis* L. (Mary Washington 500W cultivar) of the family Asparagaceae (Asparagales). Seeds were procured from Agricultural Research Center (Institution of Vegetable), Dokii, Giza, Egypt.

The cultivation was carried out in the Agric. Experiments and Res. Station, Faculty of Agriculture, Cairo University, Giza, Egypt during the two successive growing seasons of 2013, 2014 to provide the experimental plant material.

Microscopical investigations were carried out to study the anatomical structure of Asparagus plant. Plant samples were taken at flowering time during the growing season of 2014. Specimens (3-5 mm) represented different plant organs, including:

1. The main stem represented by shoot apex, terminal and median portions.
2. The cladode (cladophyll).
3. The spear.
4. The scale-like leaf.
5. The rhizome.
6. The adventitious root.

Microtechnique procedures were carried out at the Laboratory of Agric. Bot. Dept. Faculty of Agric., Cairo University, during the second season. Plant materials were killed and fixed for at least 48 hrs. In F.A.A. (10 ml. formalin, 5ml glacial acetic acid, 85ml ethyl alcohol 70%) and dehydrated in a normal butyl alcohol series before being embedded in paraffin wax melting point 56°C **(Sass, 1951)**. Sections which were cut on a rotary microtome at a thickness of 15-20 microns were stained with crystal violet / erythrosine before mounting in Canada balsam. Slides were examined microscopically and photomicrographed.

The major chemical metabolites contents of spear were determined according to the methods of **Thomas and Dutcher (1924)**, **Arnon (1949), Blight and Dyer (1959), AOAC (2000), Anon (2009a) and Ergonul and Nergiz (2010)**.

RESULTS AND DISCUSSION

1. The external feature

Asparagus is a herbaceous, perennial and dioeciously vegetable crop, growing to 1.5-2m long , with stout stems (ferns) with much-branched feathery foliage **(Fig. 1.)**. The leaves are triangular scale- like 3-7mm in length. The cladodes (modified stems) arise in the axis of scale leaves. The aerial erect shoots carry cladodes (cladophylls). The cladodes were found in fascicles (3-6) on each node (18-30mm long and about 2-3mm broad).

The flowers are bell shaped, greenish –white to yellowish, 4.5-6.5mm long. Tepals are partially fused together at the base. Flowers are produced single or in clusters of 2-3 in the junctions of the branchlets. The female flower contains a well-developed pistil and vestigial stamens, but the male flower has six developed stamens. The fruit is small red berry at maturity, 6-8 mm in diameter, which is poisonous to human. The floral formula of the disc flower is as follows:

$$\oplus \ ♀ \ p(3+3)\ A - \underline{G}_{(3)}$$

$$\oplus \ ♂ \ p\ (3+3)\ A_{3+3}\ G_{(-)}$$

The crown consists of rhizomes, (fleshy underground stems) with adventitious roots attached to their basal portion. Furthermore the crown contains the buds of nascent spears sticking up (marketable yield). At the age of 2 years – old, the diameter of the crown was about 15-20cm. For best results, crowns used for planting must be 2 years –old. The young aerial stems or spears (about 20-25 cm long) arising from rhizomes are consumed as a vegetable.

These findings are in agreement with those of **Watson & Dallwitz (1992), Nichols, (1993), Tanming & Chantaranathai (2011) Hyde et al (2012), Smith & Harbott, (2012) and Harb, et al (2015).**

2. Anatomical investigations

a. The main stem

1. shoot apex

Shoot apex meristem is usually dome shaped and arranged in two distinct zones, the outer is tunica composed of two layers of small, essentially

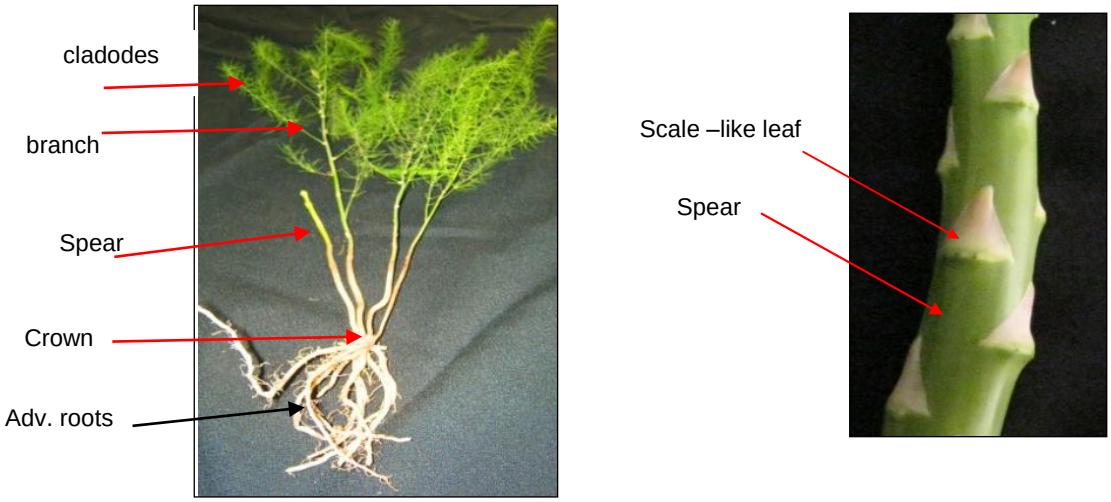

Fig.1. A Photograph of *Asparagus officinalis* L. showing the spear (marketable yield) and the vegetative growth of *A. officinalis* L.

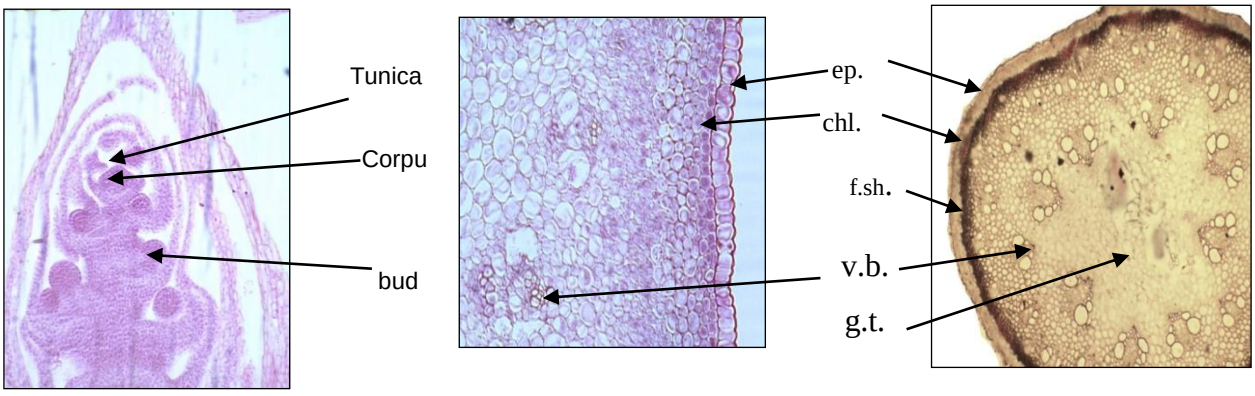

Fig. 2. Longitudinal section through the shoot apex (a); cross sections through the terminal internode (b) and the stem (median portion) (c) of *A. officinalis* L.
Details: ep ., epidermis; chl., chlorenchyma ; f.sh. , fiberous sheath ; v.b. , vascular bundle; g.t. , ground tissue .

thin – walled. The tunica is peripheral layer of cells that divided anticlinally to the surface of the meristem. The corpus is a mass of cells in the central part, the cells occur in various lines and the cells are irregular in size. The corpus cells appear to be arranged in irregular positions. Tunica responsible for initiating epidermis and other parts differentiate from corpus. Differentiate leaf primordium and stem were initiated below shoot apex as shown in **Fig. (2,a)**. It is obvious that the apex is responsible for differentiating scale – like leaf and cladodes.

2. Visible internode below the shoot apex

Transverse section of visible internode below shoot apex **(Fig. 2, b)** show that absence of fiberous sheath below epidermis. Epidermis is followed by 2-3 layers of chlorenchyma and 4-5 layers of lacunar collenchyma. Numerous vascular bundles are embedded in the ground tissue, with pith more narrower than in median internode. Small vascular bundles are outside whereas the largest ones toward the inner side. Chlorenchyma tissue featured of idioblasts with raphides located between them. Dimensions of small vascular bundle recorded thickness of (87.5µ and 81.2µ), whereas the corresponding dimensions of the large vascular bundle were 187.5 and 162.3µ.

3. The aerial stem (median portion)

Transverse section of median portion of the aerial stem **(Fig. 2, c)** shows that the stem has an oval form with uniseriate layer of epidermis, which is interrupted with stomata and covered by a thick cuticle layer recorded thickness of 12.5µ. Epidermis is followed by 2-3 layers of parenchyma tissue, then fiberous sheath in a continuous ring surrounding the stele. Fibrous sheath consists of 5-6 layers of sclerenchyma with very thick secondary walls which surrounded the vascular bundles. Through the ground tissue there are three ranks, the biggest bundles were disposed to the inside and the outside two ranks covered smaller bundles. This type of the central cylinder is known as atactostele. Dimensions of the small vascular bundle were 100µ and 112.5 µ for width and length respectively, whereas the corresponding dimensions of the large vascular bundle were 162.5, 200µ. The central cylinder was good represented by polygonal parenchymatous cells, which increased gradually in their size towards the centre, having small triangular intercellular spaces among them. These findings are in harmony with those of **Steudle (2000)**, **Ali & Khan (2009), Balasolu et al (2010) and Rodica (2011).**

b. The cladodes

It is obvious from **Fig. (3, a)** that cladode is oval in shape. The epidermis is uniseriate oval cells and showed thickness of 25µ. Cuticle layer on epidermis records thickness of 9.3µ. There are two rows of prolonged radial chlorenchymatous cells and also characterized by small area of vascular tissue. The reason of increasing mesophyll tissue (thickness of 62.5 µ) is to compensate the lack of normal leaf. The vascular bundle is surrounded by 1-2 rows of compact parenchymatous cells. Vascular bundle is featured by two alternative arms of xylem (5-7 vessels for each arm) with two strands of phloem are expressed. The previous description synchronized with **Quentin, (2009) and Raycheva & Stojanov, (2013)**.

c. Median portion of spear

Transverse section of median portion of spear **(Fig. 3, b)** shows that oval epidermis cells recorded thickness of 12.5µ which covered with cuticle, follows by 2-3 layers of chlorenchymtous cells that recorded thickness of 37.5 µ. Several layers of parenchyma cells were found and followed by condense of parenchyma cells make ring around vascular bundles, then vascular bundle diffused through the ground tissues. Small external vascular bundles observed in the outermost layer but internal area showed large vascular bundles toward the central portion. Dimensions of small vascular bundle (outside) were 87.5µ and 100µ for width and length, respectively whereas the corresponding dimensions of large vascular bundle were (inside) 187.5 and 237.5 µ. It is obvious that, vascular bundles were diffuse in all sector without central hollow as in median portion of the aerial stem.

d. The scale- like leaf

Transverse section of scale- like leaf **(Fig . 3, c)** reveals that cuticle layer is exist on lower and upper epidermis and recorded average thickness of 12.5µ and 15.6 µ for lower and upper epiderm respectively. Epidermis cells are long and the stomata are interspersed through the epidermis cells. Number of stomata in the abaxial side (lower side) is more than the adaxial one (upper side). Mesophyll (268.7µ in thickness) consists of irregular parenchyma and famous of rhaphid crystals. There are poor developed vascular bundles found in continuous line. Type of vascular bundle is collateral, xylem is found in the adaxial side whereas phloem is found in the abaxial one. These results are similar with that of **Rodica (2011)**.

e. Subterranean stem (the rhizome).

As shown in **Fig. (4, a)** transverse sections of rhizome (subterranean stem) are characterized by presence of amphivasal vascular bundle (central

phloem vascular bundle surround by xylem) embedded in parenchyma cells with thin cell wall. Epidermis layer is replacing by storied cork (multiple cortex), which partially sobereous. Dimensions of the amphivasal vascular bundle recorded average of 281.3 and 287.5 µfor width and length, respectively.

f. The adventitious root

In cross section, the adventitious root has a round form **(Fig. 4,b)** and appears to be formed of a series of concentric cylinder. The first layer (15.6 µ in thickness), called rhizoderma, was represented by small cells, some of them became absorbing hairs by elongation.

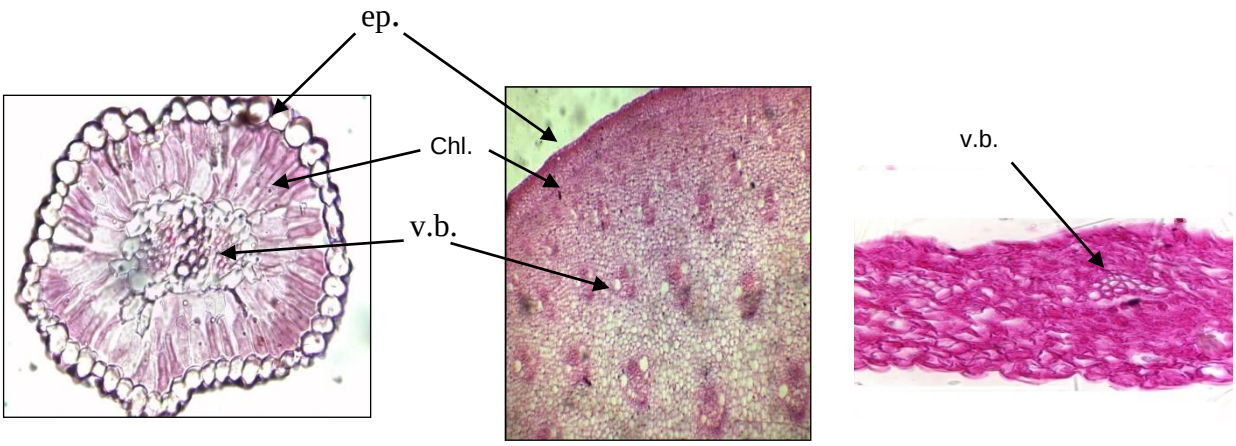

Fig. 3. Cross sections through the cladode (a) , median portion of the spear (b) and through the scale –like leaf (c) of *A. officinalis* L.
Details: ep., epidermis; chl., chlorenchyma; v.b., vascular bundle.

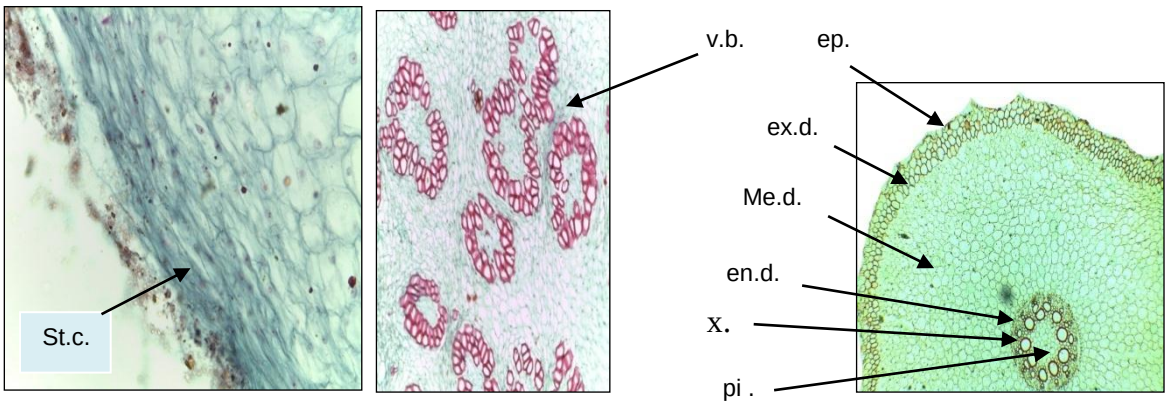

Fig. 4. Cross sections through the rhizome (a) and the adventitious root (b) of *A. officinalis* L.

Details: ep., epidermis; ex.d., exodermis; me.d., mesodermis; en.d., endodermis; x., xylem; pi., pith; St.c., storied cork.

Exodermis consists of 4-5 layers of suberificated cells (143.7µ in thickness). The cortical parenchyma had 15-18 ranks of oval cells, with intercellular spaces and these cells deposited the reticence substances. The storage adventitious roots of Asparagus being metamorphosed, more exactly tuberified.

The last layer of cortex, the endodermis, was formed from cells with lateral suberous walls thickening (Casper's strips) interrupted by passage cells.

The central cylinder includes the pericycle, the ligneous fascicles, the liberian fascicles, medullar rays and the medullar parenchyma (pith). Xylem was easy to distinguish through the lignified walls (9-12 bundles). The external vessels besides the pericycle, had a small diameter forming the protoxylem. The subsequent differentiated vessels to the root center, had a bigger diameter and integrates the metaxylem.

The Liberian fascicles are smaller, with cellulose walls form the protophloem and are adjacent to the pericycle. The fascicle that with a bigger diameter are disposed inside, shaping the metaphloem. The center of root is proved to be a parenchyma formed from round and oval cells (pith). Pith records 362 µ in diameter.

These results are in general harmony with that given by **Eschofield (1997) and Hurgoiu and Sipos (2005)**.

3. **Chemical determination**

a. The major metabolites contents

Samples of *Asparagus officinalis* L. spear were collected twice at the onset of flowering time for chemical analysis.

Percentages of the total carbohydrates, crude protein, fat, crude fiber and ash of asparagus spears are revealed in **Table (1)**. Results illustrate that the percentages of total carbohydrate, crude protein, fat, crude fiber and ash were 44.01, 10.4, 4.72, 22.90 and 10.87 %, respectively. The fat showed the lowest content, being 4.72%. On the other side, the total carbohydrate of asparagus spear recorded the highest value (44.01%).

Similar findings were found on *Asparagus officinalis* plant by **Cake and Bartlet (1922), Waldron and Selvendran (2006) and Aberoumand, (2010)**.

Table 1. Percentage of total carbohydrate, crude protein, fat, crude fiber and ash of *Asparagus officinalis* L. spear at the onset of flowering time, mean of 2 samples.

Composition of spear	Percentages (%)
Total Carbohydrate	44.01
Crude Protein	10.40
Fat	04.72
Crude fiber	22.90
Ash	10.87
Humidity	07.10

b. Total chlorophylls, sugars and thiamin

Table (2) reveals the average of total chlorophyll (a and b), total sugars (reducing and non-reducing) as well as thiamin (Vit .B_1) of asparagus spears (d.wt.).

Data show that the content of total chlorophyll was 20.01 µg/g. The chlorophyll (a) and (b) contents were 12.93, 6.57 µg/g, respectively. Also, data show that the content of total sugar was 2.28 mg /g. The contents of reducing and non- reducing sugar were 0.46, 1.31 mg / g, respectively. With respect to vitamin B_1 (thiamin) data show that the content of thiamin was 0.003 µg/g d.wt.

These results are in harmony with those of **Deputy (1993), Hassan, Neveen (2001) and Sakaguchi et al (2008)**.

Table 2. Chlorophylls, sugars and thiamin (Vit.B_1) contents of *Asparagus officinalis* L. spear at the onset of flowering time, means of 2 samples

Biochemicals	Measurements
Chlorophyll(a)	12.93 µg/g d.wt.
Chlorophyll(b)	06.57 µg/g
Total chlorophylls	20.01µg/g
Reducing sugars	00.46mg/g
Non reducing sugars	01.31mg/g
Total sugars	02.28mg/g
Thiamin (Vit. B_1)	00. 003µg/g

c. The organic acid contents

Results of the organic acid contents of *Asparagus officinalis* L. at the onset of flowering time of the corresponding chromatogram peaks are given in **Table (3) and Fig. (5)**.

HPLC analysis of spear at the onset of flowering time was known to be a complex mixture contains 6 components of organic acids. The components were designated according to their retention times for further reference .Many could be identified according to the detector response.

Results illustrate that the contents of oxalic, citric, tartaric, lactic, formic and acetic acid were 27.58, 0.22, 3.19, 90.37, 26.72 and 8.03 mg/100g, respectively. Similar findings were recorded on *Asparagus officinalis* L. plant by Stephen and **Mitchell, (2013)** and, also **Yeasmin et al (2013)** who found that organic acids have important role in the allelopathic responses of asparagus replanting problem.

Table 3. HPLC analysis of organic acids in asparagus spear at onset of flowering time

RT(min)	Contents (mg/100g d.wt.)	Org.acid
6.68	27.58	Oxalic acid
8.15	0.22	Citric acid
8.90	3.19	Tartaric acid
12.85	90.37	Lactic acid
13.47	26.72	Formic acid
14.52	8.03	Acetic acid

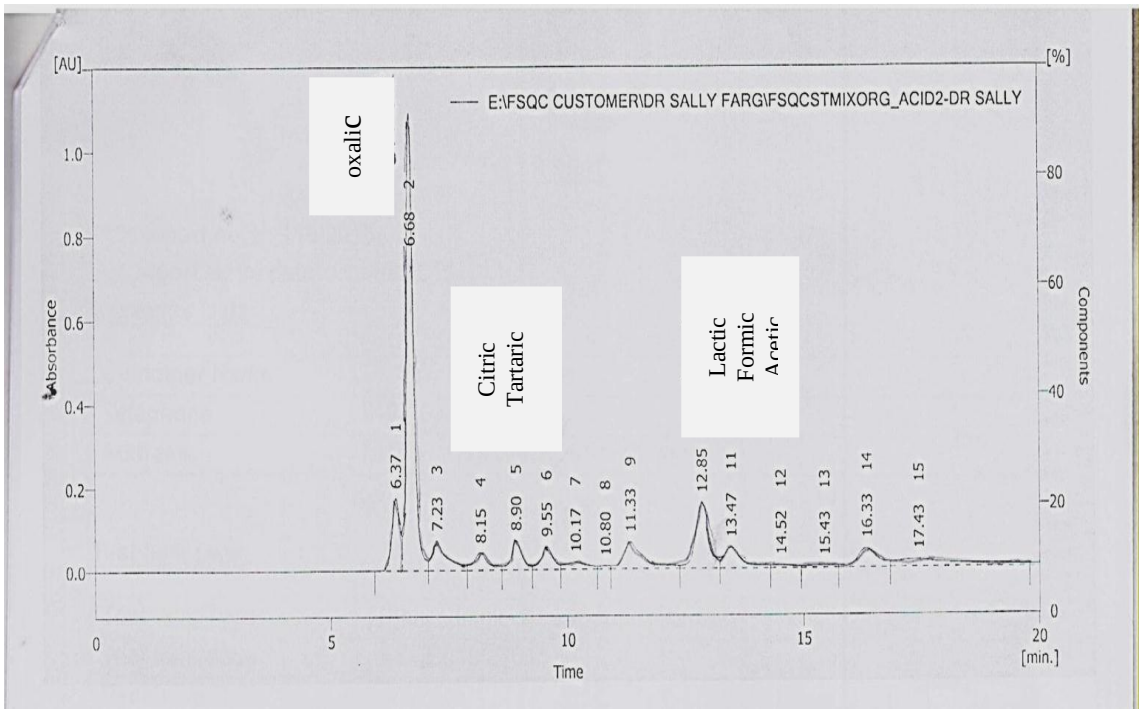

Fig. 5. High performance liquid chromatogram of organic acids of *Asparagus officinalis* L. spear

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