

## Investigation of some Carboxylic Acids and Phenolic Compounds of *Ailanthus altissima* Leaves and their its Effect on Italian Cupressus Seedlings Root Rot Fungi

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### ABSTRACT

The study was carried out for separation and identification of some carboxylic acids such as Aspartic, Citric, Tartaric, Propionic, Ascorbic, Maleic and Fumaric, Adepic acid, as well as Phenol, Resorcinol, Hydroquinone, Quercetin, p-Hydroxybenzoic, Benzoic acid and Gallic acid from *Ailanthus altissima* determined by High Performance Liquid Chromatography (HPLC). Moreover the major components of carboxylic acid were presented as Ascorbic acid (86.38%). Also the phenolic compounds was studied and the results showed that P- Hydroxy benzoic acid (41.99%) was the highest amount. Isolation results from Cupressus seedlings infected with root rot disease appearance of the fungus *F. solani*, *F. oxysporum*, *F. chlamydosporium* and *Rhizoctonia solani*, the isolation ratios was 41% as a maximum value for *F. solani*, then *F. oxysporum* (27%), *F. chlamydosporium* (12%) which was minimum value and *Rhizoctonia solani* (20%).

Bioassay results of *Ailanthus altissima* leaves extract showed an increasing inhibition ratio of fungi growth with increasing leaves extract concentrations, *F.oxysporum* and *F.chlamydosporium* had the highest degree of growth inhibition (100) % for the two fungus at (4%) extracts concentrations, then followed by the fungus *F.solani* and *Rhizoctonia solani* were (84.78,57) % respectively and *Rhizoctonia solani* had the minimum inhibition.

The results of average fungus growth treated with several concentrations of *Ailanthus* leaves extracts with PDA media showed inhibition of growth with increasing extracts concentration, *F. chlamydosporium* had minimum mean growth 7mm at 1% extract conc. whereas the two species of *Fusarium* showed maximum inhibition for average growth were zero values at 4% of extract conc. except *Rhizoctonia solani* which had a different value (19.67) mm at the same conc.

**Keywords:** *Ailanthus altissima*, Carboxylic acids, phenols, HPLC.

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التحري عن بعض المركبات الفينولية والاحماض الكربوكسيلية في اوراق لسان الطير وتأثيرها على فطريات تعفن جذور شتلات السرو الايطالي

### الملخص

تشير الدراسة الحالية الى فصل وتشخيص بعض الاحماض الكربوكسيلية كحامض الاسبارتك، الستريك، التارتاريك، البروبانويك، الاسكوربيك، المالك، الفيوماريك، وحامض الاديك، فضلاً عن المركبات الفينولية كالفينول، ريسورسينول، هيدروكينون، كورستين، باراهيدروكسي بنزويك، حامض البنزويك، حامض الكالبيك. وأشارت النتائج الى أن حامض الاسكوربيك

ظهر بأعلى نسبة بلغت 86.38 % ، وكذلك تم دراسة المركبات الفينولية وأظهرت النتائج ان حامض بارهيدروكسي بنزويك ظهر بأعلى نسبة بلغت 41.99 %.

أظهرت نتائج العزل من شتلات السرو المصابة بمرض تعفن الجذور فطريات *F. oxysporum* و *Fusarium solani* و *F. chlamydosporium* و *Rhizoctonia solani* وبلغت النسبة المئوية للعزل 41% كحد أقصى مع الفطر *F. solani* تلتها الفطريات *F. oxysporum* بنسبة 27% و *F. chlamydosporium* والذي بلغت نسبته المئوية أدنى قيمة (12%)، أما الفطر *Rhizoctonia solani* فكانت نسبة العزل 20%، وتبين من نتائج الاختبار الحيوي لمستخلص أوراق لسان الطير *Ailanthus altissima* تأثير الفطريات مع زيادة التراكيز للمستخلص حيث أظهر الفطر *F. chlamydosporium* أقصى تثبيط بتأثير المستخلص بلغ 100% عند التركيز 4% من المستخلص تلتها الفطريات *F. solani* و *Rhizoctonia solani* حيث بلغت قيم تثبيطها ( 84 و 57 و 87 ) على التوالي، في حين أظهر الفطر *Rhizoctonia solani* أدنى نسبة تثبيط بتأثير المستخلص.

كذلك أظهرت نتائج الاختبار الحيوي لمستخلص أوراق لسان الطير تثبيطاً معنوياً في متوسطات نمو الفطريات مع تركيز المستخلص حيث أظهر الفطر *F. chlamydosporium* أدنى ميوسط نمو تثبيط بتأثير مستخلص الأوراق إذ بلغ 7 ملم عند التركيز 1% من المستخلص، في حين أظهرت نوعي الفطر *Fusarium* أقصى تثبيطاً للنمو بلغت قيمته صفر عند التركيز 4% باستثناء الفطر *Rhizoctonia solani* الذي كانت قيمة متوسط نموه 19.67 ملم عند نفس التركيز.

**الكلمات الدالة:** لسان الطير، الاحماض الكاربوكسيلية، الفينولات، HPLC.

## INTRODUCTION

The plant *Alianthus altissima* commonly known as "there of heaven" or "smoke tree" belongs to the Family Simaroubeceae (Weekar *et al.*, 2017). *A.altissima* is a fast- growing deciduous tree which is native to Asia. It was introduced in to Europe (1751) and to the United States (1784) into Eastern states by a Philadelphian gardener and in to western states by Chinese immigrants who used it for medicinal purpose. However, the tree was originally indigenous to china, but today it grows the wild and is cultivated in tropical and subtropical eastern Asia, Northern Europe and North America (Masteli and Jerkovi, 2002).

*A.altissima* is used in traditional medicine as a bitter aromatic drug in the treatment of colds and gastric diseases. The plant is known to have an antimalarial activity due to presence of active chemical constituents such as indole alkaloids, lipids, fatty acid, phenolic derivatives and volatile compounds from leaves (Raja *et al.*, 2017). Monocarboxylic acids such as formic acid, acetic acid or propionic acid are fundamental materials in the chemical industry. The carboxylic acid are most widely used in the field of food and beverages as an acidulate and also in pharmaceutical and chemical industries. Carborylic acids are classified by the chemical structure R- COOH. In this acid form, they are fully hydrocarbon soluble. Only those organic acids with a carbon of five or less exhibit water solubility. However, an important characteristic of organic acids is that alkali metal salts of those compounds are readily souble in water and insoluble in hydrocarbon media (Sushil and Badu, 2014). Phenolic compounds are a group of secondary metabolites that are widespread in the plant kingdom. They are characterized by diverse chemical structure and numerous pharmacological properties. Their molecules contain two functional groups: a carboxyl group and a phenolic hydroxyl group (Carolina *et al.*, 2014). Because of chemical structure, they can be divided into derivatives of cinnamic acid benzoic acid which differ from each other in the number of hydroxyl groups and the placement of methoxyl substitution, other pharmacological effects of

phenolic acids are: antipyretic, antibacterial, anti-inflammatory, antipyretic, antifungal, anthelmintic, cholagogic and immune stimulant (Itoh, 2010).

Root rot fungi diseases is one of famous diseases and wide world pervasive which attach on tree roots cause high losses in nurseries (Agrios, 1987).

The importance of root rot diseases notice threw the many studies in world as compartment with another diseases (leaves, fruits and stems diseases) (Mohamed, 1994).

Cupressus seedlings root rot is an example of this kinds of diseases As the nurseries soil are unsterilized, the fungus, *Macrophomina phaseolina*, *Fusarium* and *Rhizoctonia solani* attached to *Pinus brutia*, Cupressus and Casuarina caused root rot disease at this seedlings (Mohammed, 1987). and the fungus *F. equiset Corda sacca*, *Macrophomina phaseolina* Tassi and seven isolations of *Rhizoctonia solani* Kuhn isolated from the roots of forest trees seedlings (Ali, 2007).

The aim of this study was the identification some carboxylic acid and phenolic compound by HPLC, and its effect on Italian cupressus seedlings root rot fungi.

#### **Taxonomic classification**

Kingdom: Plantae.  
Phylum: Spermatophyta  
Subphylum: Angiospermae  
Class: Dicotyledonae.  
Order: Rutales  
Family: Simaroubaceae  
Genus: *Alianthus*  
Species: *Alianthus altissima* (NRCS, 2012).

## **MATERIALS AND METHODS**

### **Plant Material**

Leaves of *A. altissima* were collected from healthy trees (10 years) before flowering in ... 2017 at university of Mosul/Iraq, and were dried at room temperature (25° 2C°) for one week, and kept in the dark until use.

### **Soxhlet Extraction**

Soxhlet extraction was carried out with standard apparatus for (6-8) hrs. by using 150g of dried leaves with 350 ml of hexane to achieve de-fatted depending on the method of (Harborn, 1973).

### **Extraction of Carboxylic Acids**

The leaves of *A. altissima* L. (150g) were re-extracted with 250 ml of methanol by using magnetic stirrer for 72 hrs. at 60°. The mixture was filtered and completed to 10ml in a volumetric flask with methanol (Grand *et al.*, 1988). the extract was filtered and evaporated under vacuum in a rotary evaporator at 65° until 20ml. The analysis was performed using HPLC (Shimadzu 20A), C18 column (5µm, 150 mmx 4.6mm) thermostatted at 30 C°, the mobile phase was 40 mM Na<sub>2</sub>SO<sub>4</sub>. The pH was 2.68 adjusted with methanesulfonic acid with flow rate 1.0 ml min<sup>-1</sup>, UV at 210 nm and injection volume was 5µL (Dionex, 2004).

### **Extraction of Phenolic Compounds**

After the extraction by hexane, the leaves of *A. altissima* (150g) were re-extracted with 350ml of absolute ethanol by using soxhlet apparatus for 72hrs. at 78 C°. the extract was filtered and evaporated for acid hydrolysis, with 1N HCL for 1hr in both water at 100C° and then the mixture was separated by using ethyl acetate when added to solution, two layers were shown, the ethyl acetate layer was kept for other analysis. The compounds that contain an ethyl acetate were identified by HPLC-Technique (Sahimadzu, LC, 2010 A/Japan). The column was C18 (4.6 × 240) mm at a flow- rate 1.5 ml min<sup>-1</sup>, the mobile phase consisting of acetonitrile: water (80: 20) V/V, UV at 280 nm (Al-Tkey, 2012). Measurements were made in the laboratory of Baghdad university/ College of Education for Girls.

### Identification of Compounds

Analysis of carboxylic acids and phenolic compounds were identified by comparing the retention time of the samples with those of the known standards (Fig 1, 3). The quantities of organic acids and phenolic compounds were estimated from the peak areas, injecting known amounts of the standards (Plein and Covdet, 2010).

### Isolation and Root Rot Fungi Identification

Isolation from Italian cupressus seedlings which infected with root rot diseases depending by Agnihorti (1971) as well as diseases symptoms which appear at vegetative parts, samples of cupressus roots infected by root rot diseases transferred to forest diseases laboratory/ Forestry Dept. / College of Ari. and Forestry, the diseases samples were washed under running water for 30min., small pieces of cupressus infected root seedlings were cut especially the infected zones to small pieces at 0.5 cm. then sterilized with 1% sodium hypochlorate for 3min., the species were removed from sterilization solution, washed with distilled water and dried between two sterile filter papers, then planted on sterilized petri dishes containing Potato Dextrose Agar ( PDA ) media supplied with antibiotic of streptomycin sulphate at 50mg/L conc. to prevent the bacterial growth, these pieces distributed as avarege (4 pices /petri dish), incubated at 25+2 c for 5 – 7 days

Purification of isolated fungi were made in test tubes containing Potato Dextrose slant agar as a nutrient media as we shall use it in subsequent experiments.

Fungi identification till to the genus level was made depending on the keys of (Barnett and Hunter, 2006) then to species depending on the keys of (Booth,1971) then isolation ratio were calculated.

### Bioassay of Ailanthus Extract

Alcoholic extract of Ailanthus leaves with concentrations (1%,2%,4%) were obtained from stock solution which contain phenolic compounds depending on Alyahya ( 2003) method, DMSO (Dimethoxysulphorside) as a solvent for dry extract of Ailanthus leaves, this conc. were added to sterile petri dishes and mixed with sterile PDA, in petri dishes at 5.5 cm, Inoculation of isolated fungi were made on petri dishes (5.5cm Dim.), disk of isolated fungi (4mm. Dim.) which was taken from colony edge that modern growth, three replication for each concentration of extract were made, Results calculated by measuring two mean of orthogonal diameters for fungi growth (Alyahya, 2003 ).

## RESULTS AND DISCUSSION

### Identification of carboxylic acids

The pH of mobile phase and temperature are crucial parameters. The most suitable mobile phase used for separation of carboxylic acids are aqueous water, pH was adjusted 2.68 value with hydrochloric acid at temperature 30 C°, therefore many carboxylic acids identified in *A.altissima* (Table 1) and Fig. (2).

An example of the chromatogram (R<sub>i</sub>) of the organic acids standards is given in (Table 1). Many carboxylic acids could be separated in less than 14 minutes. Among the organic acids, Ascorbic acid showed the highest value followed by citric, Aspartic, and Maleic acids.

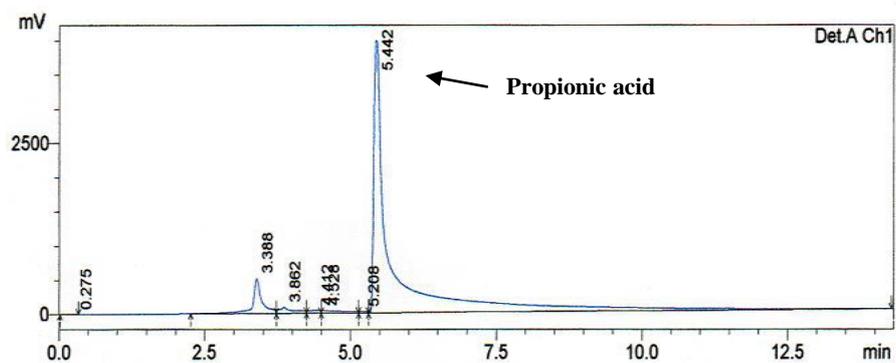
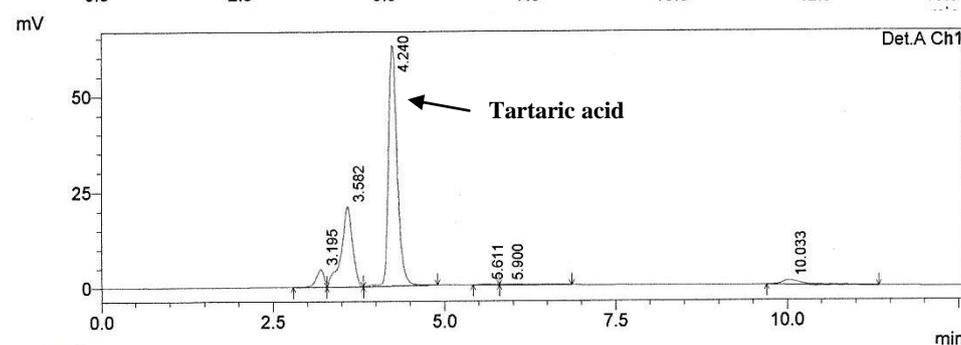
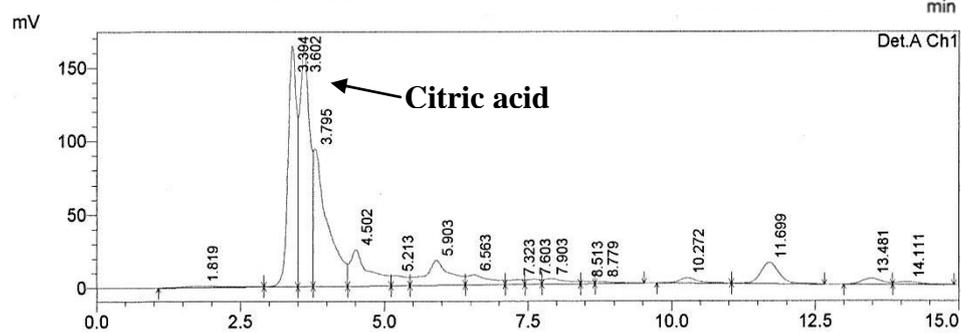
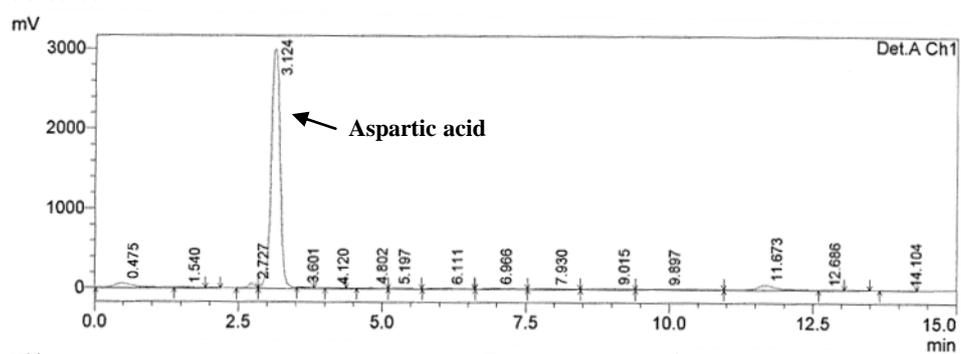
The individual and total levels of carboxylic acids in Ailanthus leaves may change according to the age of tree and the variety.

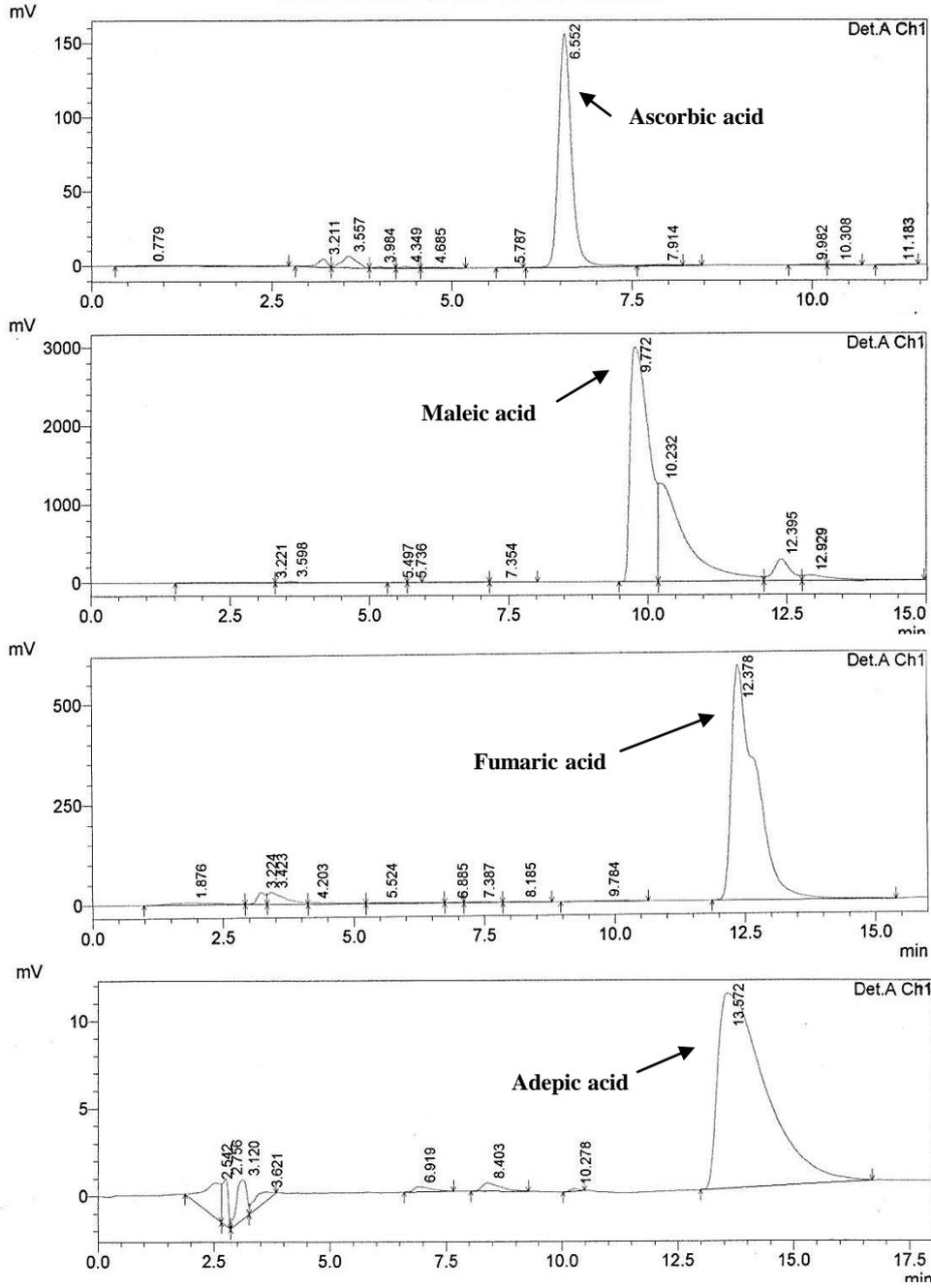
In conclusion, this methods is suitable for the determination of carboxylic acids in Ailanthus leaves. They can be determined in low concentrations with a great sensitivity. The procedure of organic acids extracts is simple and rapid These results were in agreement with the findings of (Pindla *et al.*, 2012) and (Meinhart *et al.*, 2012).

**Table 1: Chemical composition of carboxylic acids for *A.altissima* using HPLC technique**

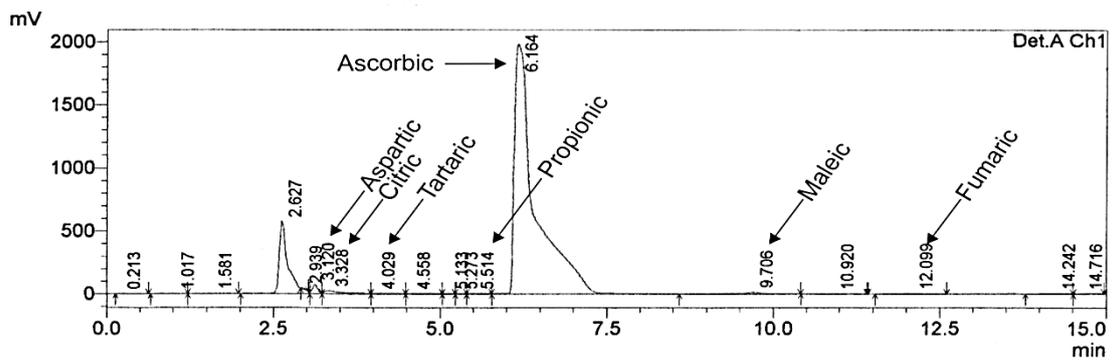
Carboxylic acids compounds	Standard $R_t$ (min)	Extract Components $R_t$ (min.)	Area%
Aspartic acid	3.12	3.12	0.841
Citric acid	3.60	3.32	0.939
Tartaric acid	4.24	4.02	0.202
Propionic acid	5.44	5.51	0.082
Ascorbic acid	6.55	6.16	86.383
Maleic acid	9.77	9.70	0.386
Fumaric acid	12.37	12.09	0.044
Adepic acid	13.57	N/D	N/D

N/D: Not detected.





**Fig. 1: HPLC chromatograms standards of carboxylic acid in *Alianthus altissima***



**Fig. 2: HPLC Chromatograms of carboxylic acid in *Ainathus altissima***

### Identification of Phenolic Compounds

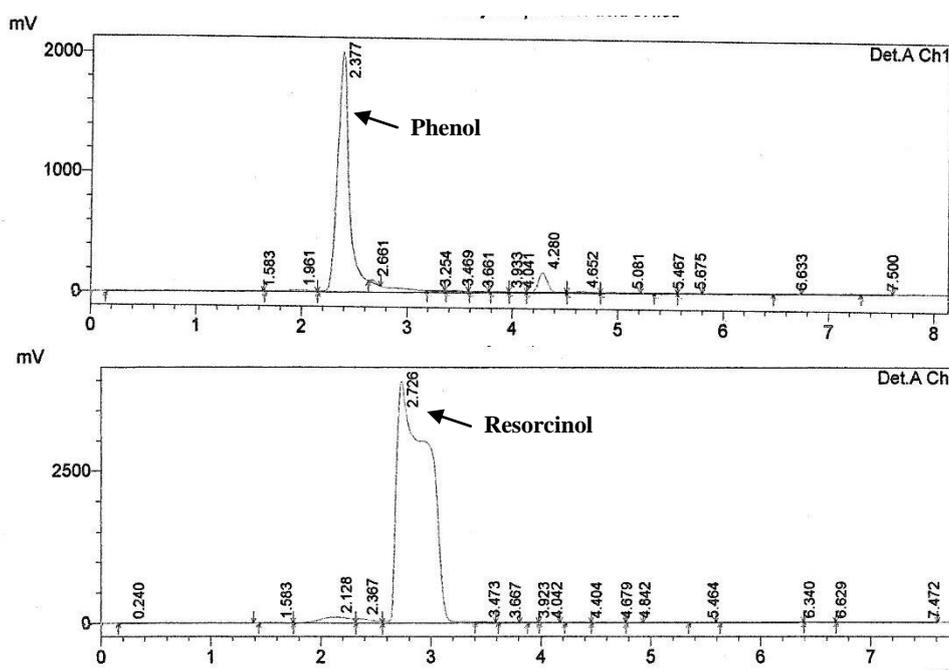
Previous studies have reported abundant Phenolic compounds in *A.altimissa*, such as (Rutin, Quercetin, Luteolin, Apigenin, Gallic acid, Chlorogenic acid, Epicatechin (Ferdaous *et al.*, 2013). Phenolic compounds play an important role for normal growth in plant development, as well as defense against infection and injury, the presence of phenolics in injured plants may have an important effect on oxidative stability and microbial safety 25\* (Plein and Cevdet, 2010), (Table 2) and Fig. (4) showed the maximum quantities (%) and retention time Rt (min) of 6 standard and sample of *A.altissima* identified in ethanolic extract by HPLC- Technique.

All phenolic compounds were identified according to their retention time and spectral characteristics against those of standards. Results confirm a variation in phenolic content of plant extracts, the highest amount of P-hydroxybenzoic Gallic acid, Quercetin appeared in extract (Shakir *et al.*, 2018) showed that the *A.altissima* leaves contain many phenolic compounds such as gallic acid, Quercetin, Rutin, chlorogenic acid, Luteolin, And a result of (Raja *et al.*, 2017) who demonstrated that the quantitative analysis showed the presence of gallic acid, coumarin, Quercetin in *A.altissima* leaves in unsound quantities.

**Table 2: Chemical composition of phenolic compounds for *A.altissima* using HPLC Technique**

Phenolic compounds	Standard R <sub>t</sub> (min)	Extract Components R <sub>t</sub> (min.)	Area%
Phenol	2.37	N/D	N/D
Resorcinol	2.72	2.67	1.648
Hydroquinone	2.83	2.98	3.767
Quercetin	3.02	3.13	9.864
P-Hydroxybenzoic	3.20	3.32	41.993
Benzoic acid	3.64	3.84	5.234
Gallic acid	4.24	4.76	22.922

N/D: Not detected



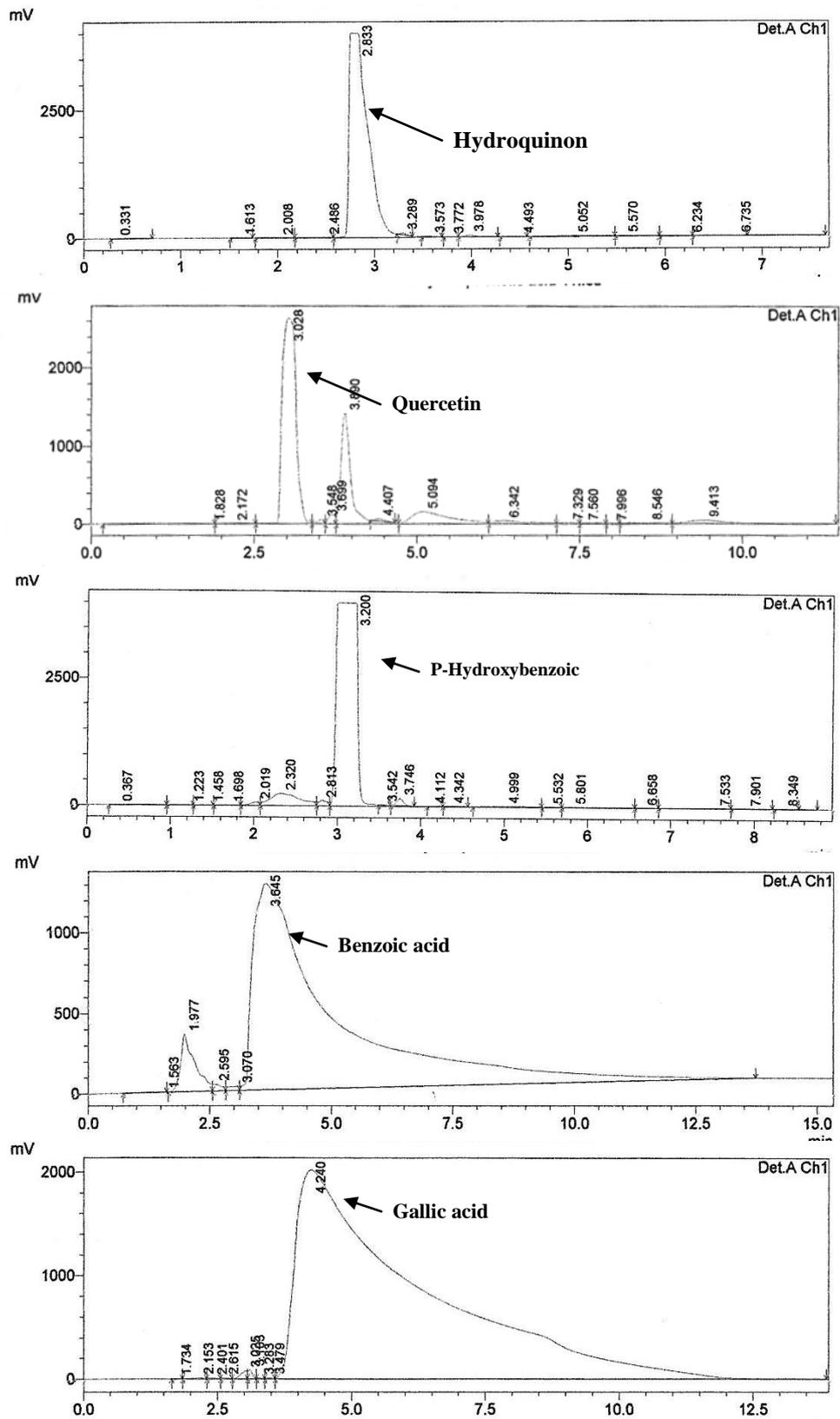


Fig. 3: HPLC chromatograms standards of phenolicsin *Alianthus altissim*

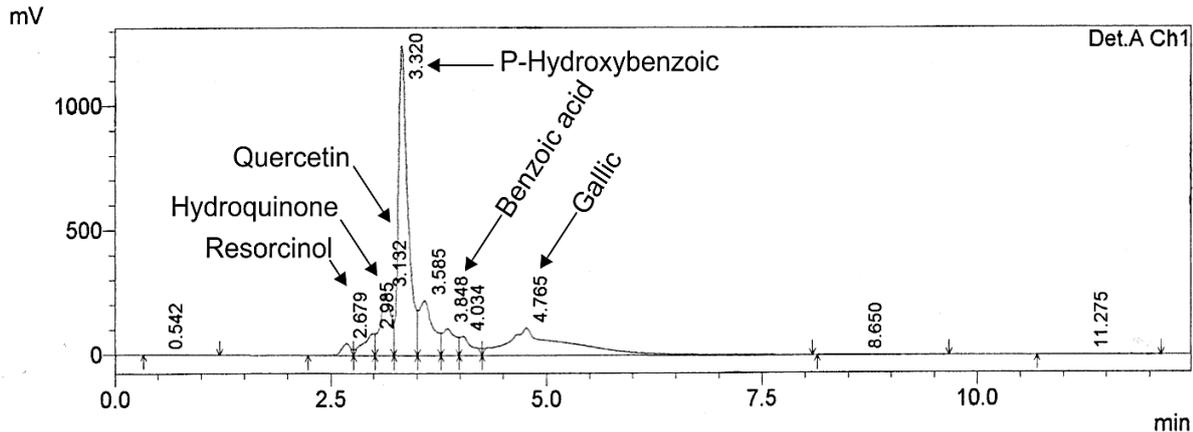


Fig. 4: HPLC chromatograms of phenolics in *Ailanthus altissima*.

#### Isolation and Root Rot Fungi Identification:

Isolation results from infected cupressus seedling by root rot disease showed appearance of the fungus *F. solani*, *F. oxysporum*, *F. chlamydosporium* and *Rhizoctonia solani* in different ratios (Table 3), the isolation ratios were 41% a maximum value for *F. solani*, then *F. oxysporum* (27%), *F. chlamydosporium* (12%) which was minimum value and *Rhizoctonia solani* (20%).

Identification of fungi were according to universal classification keys to genus level (Barnett and Hunter, 2006) and to species level (Booth, 1971).

Table 3: Fungi isolation ratio from Italian Cupressus seedling infected by root rot diseases root

Fungi	% Isolation
<i>Rhizoctonia solani</i>	20
<i>F. solani</i>	41
<i>F. oxysporum</i>	27
<i>F. chlamydosporium</i>	12

#### Bioassay of Ailanthus Leaves Extract

##### Average growth inhibition ratio calculation:

The results of bioassay for *Ailanthus altissima* leaves extract showed (Table 4) It was increasing in inhibition ratio of fungi growth with increasing of leaves extract concentrations, *F. oxysporum* and *F. chlamydosporium* had the highest degrees of growth inhibition (100) % for the two fungus at (4%) extracts concentrations, then followed by the fungus *F. solani* and *Rhizoctonia sp.* were (84,78,57) % respectively and *Rhizoctonia solani*. had minimum of inhibition.

Ethanollic leaves extract was anti fungal due to the glycoside compound and phenolic compounds which was anti fungus, the results of identification for extracts compounds by High Performace Liquid Chromotography, previous results were supported with (Ratha *et al.*, 2003) about their using the ethanol extracts of *Ailanthus altissima* leaves for stopping the growth of fungus *Aspargillus niger*, *Penicillium*, *Aspargillus flavus*, *Aspargillus fumigant*.

**Table 4: Bioassay of different concentrations of Ailanthus leaves extracts and its affect on growth inhibition ratio of Italian Cupressus seedlings root rot fungi**

Extracts Concentration Fungi	Cont.	1%	2%	4%
	Growth inhibition ratio			
<i>F.oxysporum</i>	0 f	28.33 de	42 bcd	100 a
<i>F.chlamydoespoium</i>	0 f	31.67 cde	39.67 fcd	100 a
<i>F.solani</i>	0 f	16.67 ef	54.33 b	84.78 a
<i>Rhizoctonia solani</i>	0 f	0 f	51 b	57 b

**Diameter Growth Average of Isolated Fungi Calculation**

The results of average fungus growth treated with several concentrations of ailanthus leaves extracts with PDA media showed inhibition growth (Table 5) with increasing of extracts concentration, *F. chlamydoespoium* had minimum mean growth 7mm at 1% extract conc. whereas the two species of Fusarium showed maximum inhibition for average growth ( zero values at 4% ) of extract conc. except *Rhizoctonia solani* which had different value (19.67) mm at the same conc.

As the results followed we can used Ailanthus alcohol extract as natural material in control the root rot disease of cupressus seedlings especially the fungus Fusarium species in 4% conc, of ailanthus leaves extracts.

**Table 5: Bioassay of different concentrations of Ailanthus leaves extracts and its affect on diameter growth averages of Italian cupressus seedlings root rot fungi**

Extract concentration Fungi	Cont.	1%	2%	4%
	Diameters growth averages (mm)			
<i>F.oxysporum</i>	16 de	15,67 de	11,33 ef	0 f
<i>F.chlamydoespoium</i>	10,33 ef	7 e	6 f	0 f
<i>F.solani</i>	55 a	45,67 b	25,33 f	8 f
<i>Rhizoctonia solani</i>	43,67 b	43 b	22,67 cd	19,67 cd

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