

*Short Communication*

## Identification major phenolic compounds in leaves of *Populus euphratica*

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### **Abstract**

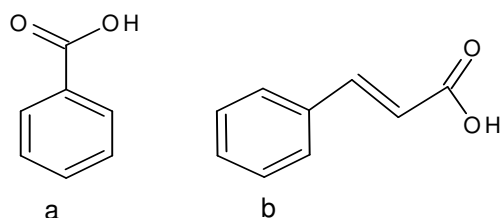
The study included the collection of samples from *populus euphratica* and prepared alcoholic and polyphenolic extracts. Used the technique HPLC for analysis the extract polyphenolic and the results showed that polyphenolic extract continue on chemical compounds Tannic acid, Caffeic acid, Comaric acid, Vanillic acid, Ferulic acid, Quercetin, Syricgeic acid by compare current results with HPLC chromatogram of standard polyphenolic compounds.

**Keywords:** Polyphenolic compounds; *Populus euphratica*; HPLC.

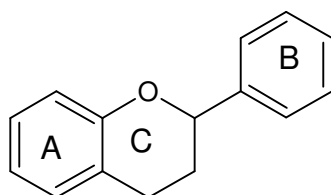
### INTRODUCTION

In order to survive in different environments, the morphological traits of widely distributed plant species often vary considerably. Leaves are exposed to aerial conditions more than any other plant organs, and the changes in their characters have been interpreted as adaptations to specific environments (Leymarie *et al.*, 1999). The ability of intra- and inter-specific hybridization within the *Populus* genus has caused the creation of a high number of sub-species and transient forms. Numerous studies have demonstrated that leaf variation is of adaptive significance for growth and competitive survival in a wide range of plants (Raschke, 1960; Parkhurst and Loucks, 1972; Hinckley *et al.*, 1989). *Populus euphratica* is a medium-size to large deciduous tree with rarely a straight stem; often bushy, but attaining a height of about 15 m and a girth of 2.5 m under favourable conditions. Bark on old stems is thick and rough, olive green, with irregular vertical figures; stem is often bent and nearly always forked; sapwood is white and broad; heartwood is reddish, often almost black at

the centre. It is shallow rooted, the roots spreading widely. Leaves are highly polymorphic; juvenile leaves 7-15 cm x 6-12 cm, narrowly oblong, usually entire; petiole 7-15 cm long; leaves on mature shoots 5-7.5 cm long, very variable, usually broader than long, rhombic or ovate, sharply lanceolate in the upper half, base 3-5 nerved; petiole 1-5 cm long, rather slender, usually with large glands at the top on either side. Research in recent years strongly supports a role for polyphenols in the prevention of degenerative diseases, particularly cancers, cardiovascular diseases and neurodegenerative diseases (Cowan, 1999). Polyphenols are strong antioxidants that complement and add to the functions of antioxidant vitamins and enzymes as a defense against oxidative stress caused by excess reactive oxygen species (ROS). Although most of the evidence of the antioxidant activity of polyphenols is based on *in vitro* studies, increasing evidence indicates they may act in ways beyond the antioxidant functions *in vivo*. Modulation of cell signaling pathways by polyphenols may help significantly to explain



**Figure 1.** Molecular structures of Benzoic (a) and Cinnamic acid (b).



**Figure 2.** Molecular structure of Flavonoid.

the mechanisms of the actions of polyphenol-rich diets (Dai and Mumper, 2010). Polyphenols are polyhydroxylated phytochemicals, which have common structures. They can be subdivided in three main subclasses, the flavonoids, phenolic acids, and the stilbenoids.

Phenolic acids are non-flavonoid polyphenolic compounds which can be further divided into two main types, benzoic acid and cinnamic acid derivatives based on C1–C6 and C3–C6 backbones (Figure 1).

Flavonoids have the C6–C3–C6 general structural backbone in which the two C6 units (Ring A and Ring B) are of phenolic nature (Figure 2).

Due to the hydroxylation pattern and variations in the chromane ring (Ring C), flavonoids can be further divided into different sub-groups such as anthocyanins, flavan-3-ols, flavones, flavanones and flavonols [Patricia *et al.*, 2010; Prior *et al.*, 2001]. This paper describes the characterization and quantification each of phenolic components in leaves of *Populus euphratica*

## MATERIALS AND METHODS

### Plants collection

*Populus euphratica* leaves were collected in Décembre 2013 from some farms in Nasiriyah city at Iraq, then it was authenticated and specimen of plant was classified in biological department-college of science at university of Thi Qar in Iraq by Asst. prof. Hayder Radhi. The leaves were cleaned, dried at room temperature for two weeks, ground as powder and kept in Dark glass containers for

further use.

### Preparation of alcohol extract

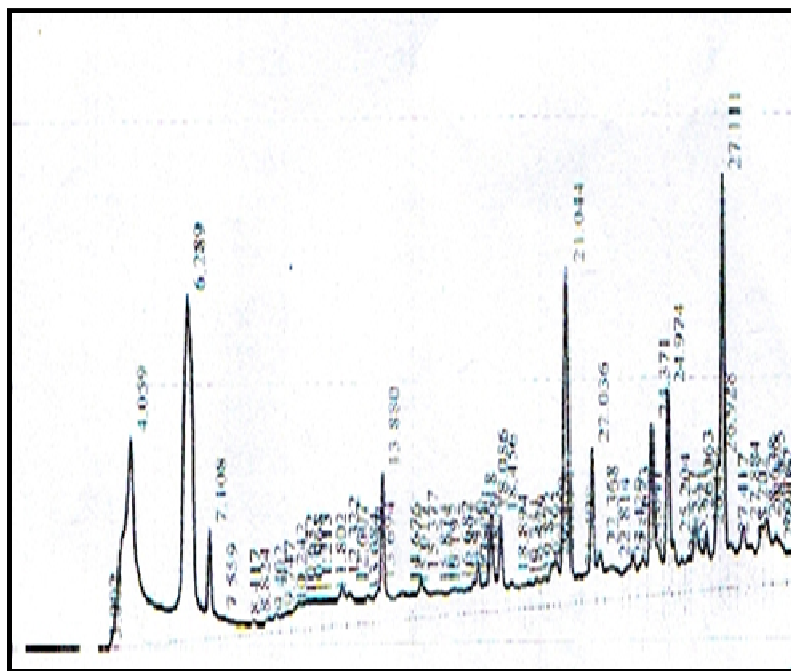
(125g) of the powder dry leaves were taken and it was (650 mL) of aqueous methanol (60% v/v) containing (1g) sodium bisulphate for (1h and 30 minutes) at (45 °C). This process was repeated 3 times to obtain enough extract for further experiments. The extract solution was filtered through Whatman No. 1 paper. The crude was concentrated using a rotary evaporator under vacuum at 40 °C. The volume of the concentrate is 200 ml.

### Extraction of polyphenols

The concentrate extracted with petroleum ether (3x100 mL) and aqueous layer extracted with ethyl acetate (3x100 mL). The organic layer contained the Polyphenols. The ethyl acetate phase was dried under vacuum.

### Acid hydrolysis

The dried of Polyphenols extract (0.5g) was dissolved in (25mL) MeOH and refluxed with (25%) HCl (10 mL) for 2h at (90 °C) and then evaporated to dryness. The dried residue was diluted with water and extracted with EtOAc three times. The EtOAc layer was evaporated to dryness and the residue was dissolved in (2mL) of MeOH. The solution was filtrated through a syringe filter.



**Figure 3.** HPLC chromatogram of standard polyphenolic compounds.

**Table 1.** Concentration of standard polyphenolic compounds.

Seq.	Polyphenolic contents in the standard	Retention time (min)	Area	Conc. Of phenolic compounds $\mu\text{g/mL}$
1	Tannic acid	6.20	1904950	25
2	Caffeic acid	7.10	301762	25
3	Comaric acid	13.88	357530	25
4	Vanillic acid	18.08	228132	25
5	Ferulic acid	18.45	228132	25
6	Querecetin	21.04	853035	25
7	Syringeic acid	24.37	468027	25

### HPLC analysis

The aglycones from hydrolysis products of Polyphenols extract was separated on HPLC (High performance liquid chromatography) column, ODS C-18 (150×4.6 mm i.d), 5 $\mu\text{m}$  practical size. The mobile phase 0.01M phosphate buffer: acetonitrile (80:20 v/v), flow rate 1 mL/min. The chromatograms were recorded at 254 nm.

## RESULTS AND DISCUSSION

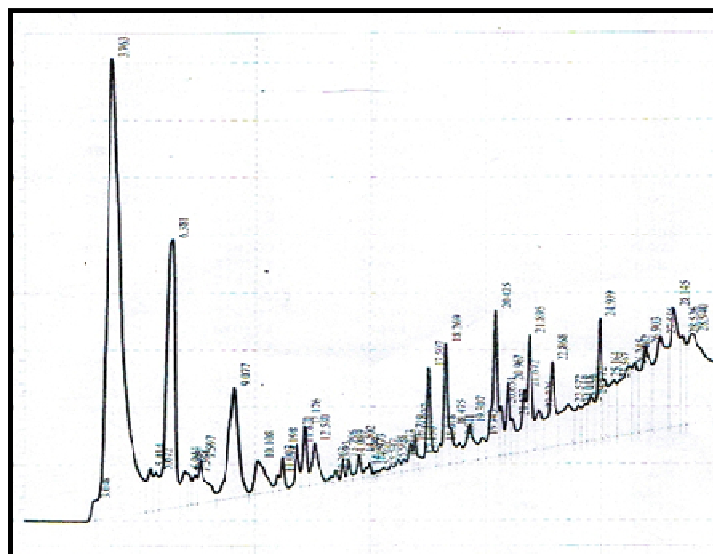
### Determination of polyphenol compounds by HPLC technique

High performance liquid chromatography (HPLC) result for sample of polyphenols are explained present of some important compounds ( tannic acid, caffeic acid, comaric

acid, vanillic acid, ferulic acid, querecetin, syringeic acid) as shown in figure (2). The peaks of the mentioned chromatogram also pointed to presence of some unknown compounds that are thought represent derivatives of polyphenolic compound.

High performance liquid chromatography is the most widely employed chromatographic technique in polyphenolic compounds analysis (Shylaja *et al.*, 2008). It has added a new dimension to the investigation of polyphenolic compounds in extract, the ability to obtain both qualitative and accurate quantitative data in one operation and the great speed of analysis (Poucheret *et al.*, 2006)

The extract was analyzed to estimate their contents of polyphenol compounds. The identification of each compound was based on retention time in comparison with pure commercial standards (Shindalkar *et al.*, 2005). Figure (3) and figure (4) explained that the retention time



**Figure 4.** HPLC chromatogram of polyphenolic compounds in leaves of *Populus euphratica*.

**Table 2.** Concentration of polyphenolic compounds in polyphenolic Extract.

Seq.	Polyphenolic contents in the standard	Retention time (min)	Area	Conc. Of phenolic compounds $\mu\text{g/mL}$
1	Tannic acid	6.3	2932966	38.49
2	Caffeic acid	7.09	155016	12.84
3	Comaric acid	13.79	89954	6.28
4	Vanillic acid	18.01	135168	14.81
5	Ferulic acid	18.47	171357	18.77
6	Querecetin	21.17	194209	5.69
7	Syringic acid	24.33	222868	11.90

of sample agrees with the retention time of the standard for most contents in each extract, and the table (1) and (2) explained the retention time of standards and polyphenolic extract respectively.

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