Identifying phenolic compounds in some genera belonging in the Amaranthaceae family by HPLC technique

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ABSTRACT

Five genera belong to Amaranthaceae family were Amaranthus albus, Amaranthus retroflexus, Alternathera sessiles, Amaranthus spinosus, and Iresine herbstis were examined by high-performance liquid chromatography (HPLC) to know the phenolic compounds. The Vanillic acid and p-coumaric acid were reported a highest concentration compering with phenols compounds under study were (795.85, 761.02 µg/ml) in Amaranthus retroflexus and Amaranthus spinosus respectively, while Protocatechuic acid founded lowest concentration compering with other phenols compounds specially in Iresine herbstis was (12.069 µg/ml), on the other hand reflect the genus Amaranthus spinosus greatest concentration of phenols compounds, whereas Iresine herbstis had a poorest concentrations from phenols compounds under study. The results gained from this study can be used as chemotaxonomy key to isolated genera, as well as to determinant of some phenols compounds it can be useful source in pharmaceutics propose. It’s worthy to mention this word is the previous chemical work to identify phenols compounds in some genera of Amaranthaceae in mid and south of Iraq by (HPLC).

1. INTRODUCTION

The Amaranthaceae are richly represented in the tropics and subtropics regions (Alonso and Crespo, 2008; Bertin, et al,2014). A particular characteristic of Amaranthaceae is a consistent presence of an exclusive group of chemical compounds such as alkaloids and phenols which have been isolated from the plants of all the genera of this family, thus it has medical attention addition used as a salad (Kim et al, 2011). Phenolic compounds which has a vast spread in the plant kingdom that is usually found in the leaves, flowers, seeds and fruits. phenolic constituents which represent the more secondary metabolic compounds They include a large number of multiple compounds, the chemical compounds are not products of redundant, but are of great importance to the scientists who found that it has evolved and specialized with the development and specialization of plants and their parts.

Chemical data obtained from chemotaxonomy may give us a good evidences to other topics like data from morphological and anatomical study, it can provides a hard foundation for a taxonomical decisions and a chemotaxonomy tool to solve many morphological issues (Radford et al,1974). The chemical analysis has been diagnosed and serve as a classifying device to separation species and taxa (Fadi et al,2012).

The determinates phenolic acids by using HPLC in different taxa in Amaranthaceae (; Lu et al, 2010; Kim et al, 2011) or the phenolic structural characterization of the isolated compounds was carried out by nuclear
magnetic resonance (NMR) (Kim et al, 2011). It is good to mention that is no HPLC method has been reported for identify of phenolic compounds in Amaranthaceae species in Iraq. Many phenolic acids such us Gallic acid has potential health-promoting effects as antioxidants and antimutagenic, anti-inflammatory and anticarcinogenic factors (Park et al, 2006 ;Wang et al, 2013).

The determination the quantitate of phenols compounds is the purpose of this study in leaves of 5 genera belong to Amaranthaceae family that may be useful for I. Chemotaxonomy’s key to isolated genera, II. Identify concentration of some phenols compounds it can be useful in Pharmacia propose, and III. Source for a future studies as bioactive compounds.

2. MATERIAL AND METHODS

2.1. “Plants materials” young fresh leaves of 5 genera were collected in October 2016 from plants that grown in different area of Baghdad. Made it a powder after dried.

2.2. “Phenols Extraction and Analysis” 10 mg of samples dissolved at 10 ml methanol HPLC grad were put in ultra- sonication (Branson sonifier, USA) at 60 % cycles at 25°C for 25 min followed by centrifugation for 15 min at 7.500 rpm. the clear supernatant of sample was subjected to charcoal treatment to remove pigments under vacuum ( Buchi Rotavapor Re type). Dried samples were resuspended in 1.0ml HPLC grade methanol by vortex, the mixture were passed through 2.5 µg disposable filter and stored at 4°C, each sample 20µg injected into HPLC system based the optimum separation condition. All chemical used were at least analytical grade, trifluoroacetic acid, phenolic acids standards were purchased from Sigma- Aldrich (Steinheim, Germany). The main compounds were separated on Fast Liquid Chromatographic (FLC ) column at optimum condition, column: pheomenex C-18, 3 μg particle size(100x4.6 mm I.D) column, Mobile phase: linear gradient of solvent A was 0.1 % trifluoroacetic acid (TFA) in deionized water; solvent B was acetonitrile gradient program for 10 minutes from 0% B – 100%B. Flow rate: 1.4 ml/min. and UV at 280 nm. The sequences of eluted material of the standard were I the figure 1, concentration of phenols compounds in the sample were calculation as µg/ ml= area of sample/ area of standard x conc. Of standard x dilution factor (Lee et al, 2007). The separation were by liquid chromatography Shimadzu 10AV- LC equipped with binary delivery pimp model LC-10A Shimadzu, the eluted peaks were monitored by UV0Vis 10A-SPD spectrophotometer (Al- Farsi, and Chan, 2008).

3. RESULTS

This study diagnosis of 8 compounds belonging to phenolic groups different based on what is an available phenols component in the genera under study as shown in Table 1. and Figure 1.

The result have been shown clear differences in the compounds levels, the genus Amaranthus spinosus had a highest concentration from 4 phenols compounds, while the genus Iresine herbstis had a lowest concentration from 4 phenols compounds.

Gallic acid record the highest abundance was (175.806 µg/ml) in Alternathera sessiles, and lowest concentration in Amaranthus retroflexus was (62. 426 µg/ml). All results in Figure 1.

Protocatechuic acid in this study was highest concentration in Alternathera sessiles was (269.465 µg/ml), but less concentration in the Iresine herbstis was ( 12.069 µg/ml).

Vanelic acid reported highest concentration in all phenols compounds under study was (795.85µg/ml) in Amaranthus retroflexus, but
in the *Amaranthus albus* have been lowest concentration was (29.80 µg/ml).

Syringic acid was strong presence in *Amaranthus albus* was (336.204 µg/ml), while in *Iresine herbstis* was fewness amount was (62.482 µg/ml).

Reported Epicatechin powering range in *Amaranthus spinosus* was (358.568 µg/ml), but in *Iresine herbstis* was (13.130 µg/ml).

P-coumaric acid, indicating the highest concentration in *Amaranthus spinosus* was (761.020 µg/ml) while the lowest concentration in *Iresine herbstis* was (88.103 µg/ml).

Quercecyin which reside in the taxa under study reached its highest concentration in *Amaranthus spinosus* was (442.908 µg/ml), but in *Amaranthus albus* was (97.644 µg/ml).

Reached leuteolin in highest level in the *Amaranthus spinosus* was (464.168 µg/ml, but in *Alternathera sessiles* was (34.113 µg/ml).

4. DISCUSSION

All genera under study share feature associate of evolutionary inter-genera of the family and this indicates it is may be can separate or isolate taxa taxonomically from each other, depending on the presence of phenolic compounds and their concentrations.

It has been found that the compounds is a phenomenon of evolutionary aspect of importance (Yasir et al, 2016).

our result of Protocatechuic acid concentration against Bertin, *et al* (2014) that cleared that protocatechuic acid was found in trace amounts in genera *Achyrantes aspera, Aerva javanica,* and *Sarcocornia,* this ascribe to different taxa in them study, but on the other hand it’s consider an evolutionary evidence with *Iresine herbstis* via his rare concentration.

Vanelic acid result agree with Bertin, *et al* (2014) they found the amount of Vanelic acid in *Sarococorina* genus (*Amaranthaceae*), this is may be referring to evolutionary relationships among *Amaranthaceae* taxa.

The outcome of Syringic acid agree with Bertin, *et al* (2014) they detected syringic acid presence with aerial parts, this is referring that Syringic acid common phenols compounds among *Amaranthaceae* family.

CONCLUSION

This study clearing indication of the ability of using of chemical compounds in isolation taxa, especially when there are overlapping in the phenotypic study, more difficult to be separated.

These phenols evidence consider a good role to help or support the morphological evidences (Fadi *et al*, 2012).

| Seq | Phenol compound | Retention time (minute) | Area µ volt |
|-----|-----------------|-------------------------|-------------|
| 1.  | Gallic acid     | 1.17                    | 214012      |
| 2.  | Protocatehuc acid | 2.06                  | 405952      |
| 3.  | Vanelic acid   | 3.99                    | 386918      |
| 4.  | Syrigic acid   | 6.06                    | 406748      |
| 5.  | Epicatechin    | 7.25                    | 411177      |
| 6.  | P-coumaric acid | 7.99                    | 294137      |
| 7.  | Quercecyin     | 8.90                    | 355633      |
| 8.  | leuteolin      | 9.80                    | 435303      |

![Graph A](imageA.png)

![Graph B](imageB.png)
Figure 1. Phenols in the some Amaranthaceae genera: A. Alternathera sessiles, B. Amaranthus albus, C. Amaranthus retroflexus, D. Amaranthus spinosus, and E. Iresine herbstii

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