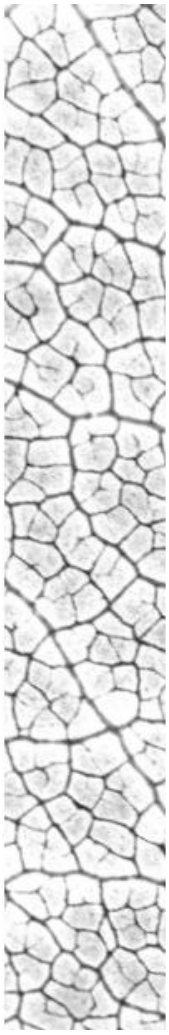

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Profiling of phenolic compounds in the grains of the genus *Secale* L. using HPLC technique

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Abstract

Background: *Secale* is an annual or perennial grasses from the Poaceae family represented in Iraq by three wild species, from which Rye (*S.cereale*) grew as a weed in Wheat fields. Rye represented a grain crop in the world used for different purposes such as alcoholic beverages, food, grazed forage. **Methodology:** This paper outlines the differences in concentrations of 10 different compounds determined by HPLC technique in three species of the genus *Secale* L. in Iraq. The content of most compounds are variable in the species. **Results:** High content of Gentisic acid are recorded in *S.montanum* Guss. and *S.cereale* while less content are observed for Luteolin-7-glucopyranoside. *S.afghanicum* (Vav.) Rozhev. reveals high content of Coumaric acid and less content of Anthocyanins. So the results indicate that the three species of the genus *Secale* represent a valuable source of biologically active constituents which can enhancing the separation of species by means of chemotaxonomic techniques. **Conclusion:** The situations of differences in concentrations of phenolic compounds play an important role in identification and separation between species studied which can be easily used as a chemotaxonomic tool.

Keywords: *Secale*, HPLC technique, chemotaxonomy

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INTRODUCTION

Secale is an annual or perennial grasses from the Poaceae family represented in Iraq by three wild species, from which Rye (*S.cereale*) grew as a weed in Wheat fields (Bor 1968). Rye represented a grain crop in the world used for different purposes such as alcoholic beverages, food, grazed forage (Oelke et al. 1990, Birhan et al. 2018).The main chemical constituents of the Rye grains has been determined using different techniques (Crausgruber et al. 2004, Fišteš et al. 2014, Hansen et al. 2004, Kan 2015, Zielinski et al. 2007) of starch, dietary fiber, protein, mineral matter in addition to antioxidants and vitamins (Jacobs et al. 1998, Mckeown et al. 2002). The natural products of plants are biologically active substances which have been provide health benefits, beyond basic nutrition to reduce the risk of several chronic diseases (Liu 2007), thus can be used in pharmaceutical and agrochemical industry (Ingle et al. 2017). Even though the reveal of phytoconstituents of wild plants have less efforts in contrast to medicinal or economic plants and this may be contribute to their remoteness from direct needs of man. Phenolic compounds are one of the phytochemicals contained in cereals grains and have antioxidants properties and can protect against degenerative diseases (Călinoiu and Vodnar 2018). The chemical structure of phytochemicals is often specific and restricted, hence useful in classification (Singh 2016) such as the presence of phenols avenanthramides in Oat (Collins 1989) and 3-deoxyanthocyannins in Sorghum (Awika et al. 2004, Gous et al. 1989).The chemical characteristics is most valuable in plant taxonomy the main purpose of this study gives an overview of phenolic compounds presence in *Secale* species and compare their content in the three species studied (Carmen et al. 2018).

MATERIALS AND METHODS

Analysis of Flavonoids

The main compounds are separated on FLC (Fast Liquid Chromatographic column under the optimum codition.

Column: phenomenex C-18, 3 µm particle size (50 x 2.0) mm I.D) column.

Mobile phase: linear gradient of, solvent A 0.1% formic acid: solvent B is (6:3:1, v.v) of acetonitrile: methanol: 0.1% formic acid, gradient program from 0%B for 10 min.

Flow rate: 1.2 ml/min.

Concentration of sample is estimated as follows:

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Table 1. The sequences of the standard eluted material

Seq.	subjects	Retention time (min.)	Area (µvolt.)
1	Salicylic acid	1.13	17280
2	Kaempferol	1.79	36292
3	Gentisic acid	2.53	33425
4	Anthocyanins	3.19	28855
5	Chlorogenic acid	4.27	35001
6	Coumaric acid	5.38	21568
7	Ferulic acid	6.10	28966
8	Luteolin	7.10	34833
9	Rutin	7.87	33686
10	Luteolin-7-glucopyranoside	8.80	31356

* standard concentration is 25 µg/ml.

Table 2. Contents of phenolic compounds in the species of the genus *Secale*

species	Concentration (µg/ml)									
	Salicylic acid	Kaempferol	Gentisic acid	Anthocyanins	Chlorogenic acid	Coumaric acid	Ferulic acid	Luteolin	Rutin	Luteolin-7-glucopyranoside
S.afghanicum	14.42	6.11	5.84	4.75	40.02	61.16	43.39	33.24	17.41	15.66
S.cereale	56.76	19.20	79.35	68.81	15.75	33.22	25.29	17.03	31.67	9.76
S.montanum	49.6	35.64	56.84	37.48	11.31	11.82	7.21	3.69	14.89	3.51

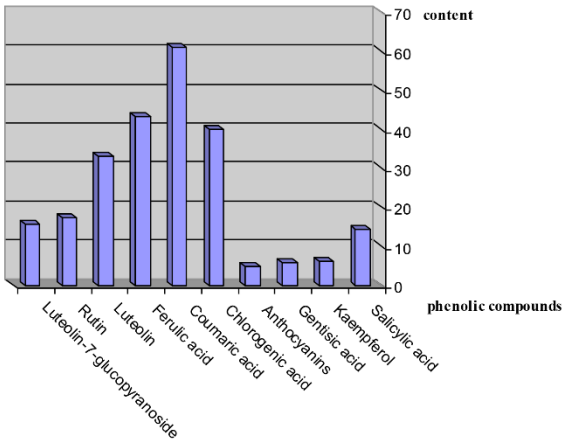

Fig. 1. Variation in phenolic compounds content in *S.afghanicum*

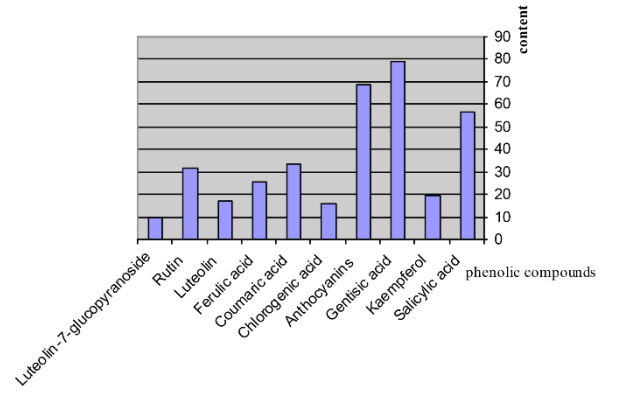

Fig. 2. Variation in phenolic compounds content in *S.cereale*

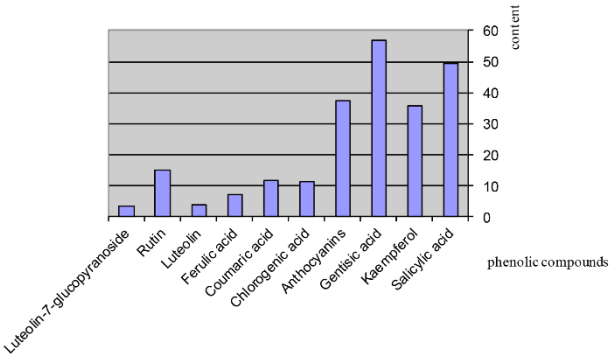

Fig. 3. Variation in phenolic compounds content in S.montanum

Concentration of sample (µg/ml)= area of sample/area of standard x conc. of standard (25 µg/ml) x dilution factor (1).

The separation occurred on liquid chromatography Shimadzu 10AV-LC equipped with binary delivery pump model LC-10A shimadzu, the eluted peaks are monitored by UV-Vis 10A-SPD Spectrophotometer.

Extraction of Phenolics and HPLC Analysis

About 0.25 of wet samples is crushed in small pieces in paste-mortar. fine-crused sample is suspending into 5 ml of ethanol-water (80:20, v/v) in glass tubes. The suspension is subjected to ultra-sonication (Branson sonifer, USA) at 60% duty cycles for 25 min. at 25 ºC followed by centrifugation at 7500 rpm for 15 min. The clear supernatant of each sample is subjected to charcoal treatment to remove pigments prior to evaporation under vacuum (Buchi Rotavapor Re Type). Dried samples are re-suspended in 1.0 ml HPLC grade methanol by vortexing, the mixture are passed through 2.5 µm disposable filter, and stored at 4 ºC for further analysis, then 20 µl of the sample injected into HPLC system according to the optimum condition.(Lim et al. 2017)

RESULTS AND DISCUSSION

The results of chemical analysis are presented in **Tables 1** and **2** and **Figs. 1-4** which have offered the presence of phenolic compounds (i.e., phenolic acids and flavonoids) in grains of *Secale* species. Phenolic

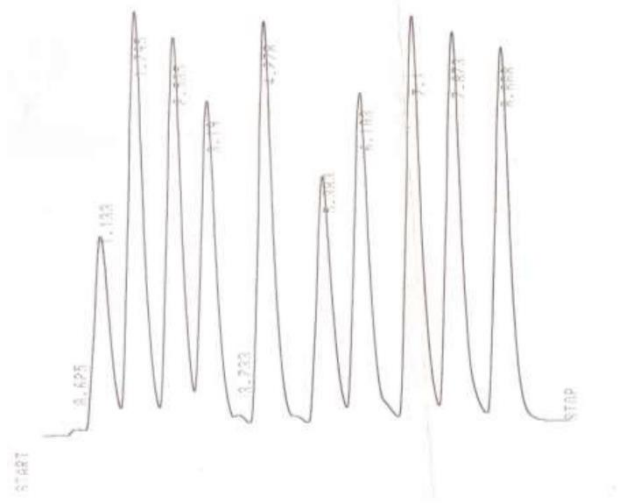

Fig. 4. Standard HPLC analysis of phenolic compounds in the grains of *Secale* species the standard concentration is 25 µg/mi each

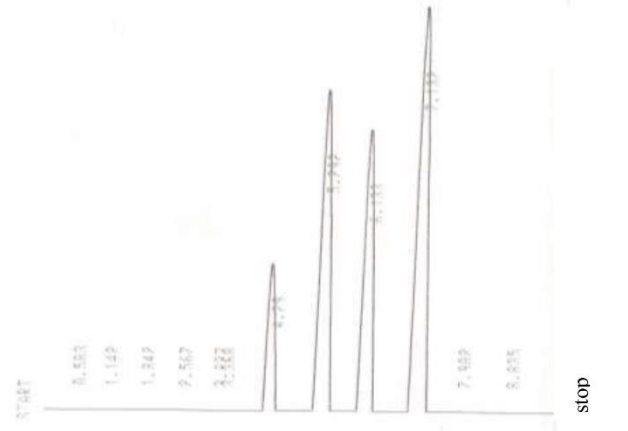

Fig. 5. HPLC analysis of phenolic compounds in the grains of *S.afghanicum*

acids are of two classes: hydroxybenzoic acids and hydroxycinnamic acids, and occur in both free or bound forms. Free phenolic acids are located in the outer layer of the pericarp (Hahn et al. 1983, 1984, Mattila et al. 2005, Sosulski et al. 1982, Subba Rao and Muralikrishna 2002). Bound phenolic acid are esterified to cell walls (Hahn et al. 1983, 1984, Kim et al. 2006, Mattila et al. 2005, McDonough et al. 2000, Sosulski et al. 1982, Zhou et al. 2004) like ferulic acid which is esterified to arabinoxylan in the cell wall (Poutanen et al. 2014). Hydroxybenzoic acids include Gentisic acid and Salicylic acid. Gentisic acid attained higher concentration in *S.cereale* and *S.montanum* (79.35 and 56.84 respectively) and less content in *S.afghanicum* (5.84). In contrast Salicylic acid have 56.77 and 49.6 in *S.cereale* and *S.montanum* respectively while it have less concentration 14.42 in *S.afghanicum.* The hyroxycinnamic acids include Ferulic acid, Coumaric acid Chlorogenic acid, Ferulic acid ranged from 43.39 in *S.afghanicum,* 25.29 in *S.cereale* and 7.21 in

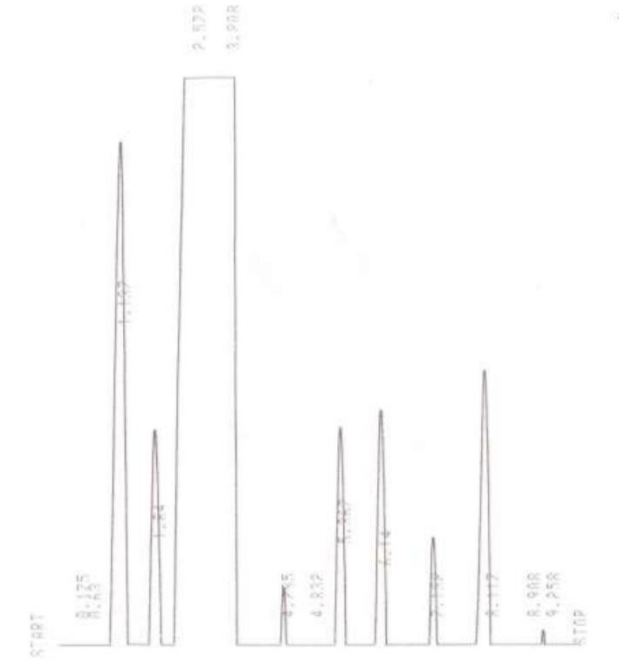

Fig. 6. HPLC analysis of phenolic compounds in the grains of *S.cereale*

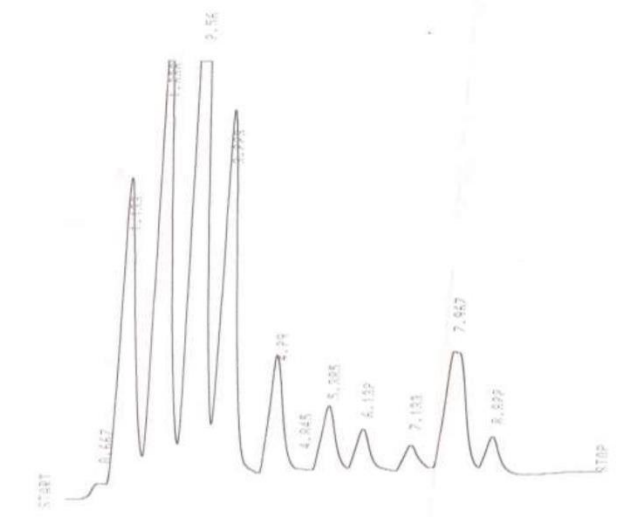

Fig. 7. HPLC analysis of phenolic compounds in the grains of *S.montanum*

S.montanum. Poutanen *et al*., 2014 have mentioned ferulic acid at highest concentration in *S.cereale* but our study reported this in *S.afghanicum.* Coumaric acid have different concentrations in the three species also with higher content in *S.afghanicum* 61.16 and 33.22 in *S.cereale* while the less content 11.82 is reported in *S.montanum.* In contrast Chlorogenic acid have convergent concentrations in *S.cereale* and *S.montanum* ranging from 15.75 and 11.31 respectively while high content 40.02 reported in *S.afghanicum.* Flavonoides in *Secale* species include Anthocyanins, Flavones and Flavonoles. Anthocyanins are water-soluble pigments and are the major flavonoides studied in cereals (Dykes and Rooney 2007). Anthocyanins

have been reported in the three species ranged from 68.80 in *S.cereale*, 37.48 in *S.montanum* and 4.75 in *S.afghanicum*. The flavonone Luteolin vareied from 33.24 in *S.afghanicum*, 17.03 in *S.cereale* and 3.69 in *S.montanum.* Luteolin-7-glucopyranoside is another type of flavones ranged from 15.66 in *S.afghanicum,* 9.76 in *S.cereale* and 3.51 in *S.montanum.* The flavonoles Kaempferol is also found in *S.montanum* with a concentration of 35.64, in *S.cereale* with a content of 19.20 and less content 6.11 in *S.afghanicum.* The study also reported Rutin which represented a glycoside of quercetin having trimeric heterocyclic structure in the species studied with a range from 31.665 in *S.cereale,* 17.41 in *S.afghanicum* and 14.89 in *S.montanum.* The flavone Luteolin have been also reported in cereals such as Parsley and Celery (Rice et al. 1997, Yao et al. 2004) our study get it in *Secale* species with a content of 33.24 in *S.afghanicum,* 17.03 in *S.cereale* and 3.69 in *S.montanum.*

CONCLUSION

The situations of differences in concentrations of phenolic compounds play an important role in identification and separation between species studied which can be easily used as a chemotaxonomic tool.

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