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Phytochemical Study of the Geranium L. in Kurdistan Region-Iraq

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ABSTRACT

Phenolic compounds of Geranium L. species were studied taxonomically by using the High Isolation and Characterization of Phenolic Compounds by Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC). Depending on the quantity and quality of the phenolic compounds the species were divided into four groups. In this study 14 phenolic compounds have been found, 9 of them were detected by using HPLC and other 5 compounds by using TLC.

Keywords: Chemotaxonomy, Geranium L., Kurdistan -Iraq

1. Introduction

Plant chemotaxonomy is the contemporary and rapidly expanding areas of plant taxonomy [1]. In fact, chemotaxonomy has various old history origins, perhaps foremost comes the search by herbalists and pharmacologists for drugs that have involved in the buildup of information about the chemical content of a very wide range of plants. The second ancient origin of chemotaxonomy are the fields of morphology and anatomy. For example, color, crystals and starch molecules which they are different in their morphology and chemical composition [2].

According to a study by Stace [2], the first study about the relationship between chemistry and plant taxonomy is the study of Abbot in 1886 on the classification of saponin in plants and then the study of Reichert (1916-1919) on carbohydrates in plants. This reasonably well known today [3,4]. According to Hegnauers dictionary of plant chemistry at least 55 species have been investigated chemically [5,6]. The *Geranium's* leaf flavonoids are typical of the Geraniaceae and correlated with dicotyledonous families. They are predominantly flavonols and the commonly contain quercetin [7]. In addition, other study by Bate-Smith [8] studied the distribution of flavonol aglycons and proanthocyanidins compounds in *Geranium*, his results revealed that all 60 tested species contain kaempferol and quercitin with various quantities which emphasize the strong relationship among these species.

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2. Materials and methods

2.1. Total Antioxidant Capacity Assay

Harborne method [9] was followed for extraction of phenolic compounds [10,11]. Leaves and stems of flowering plants were grinded by electric grinder. Then, 40-50 ml of alcohol 70% was added to 3-4gm of each specimen, and left at room temperature for 24-48 hrs. After that, infiltration was done by filter paper (ederol medium pore filtering). The extract was concentrated to adequate volume in order to get rid of alcohol by using air conditioner. Subsequently, as much as volume of Petroleum Ether (80-100 boiling point) was added to the product. Then, the mixture shacked gently placed in separating funnel and left for three minutes to separate clearly into two layers. Thereby the major part of chlorophyll dissolved in petroleum ether, and float. The extract were concentrated approximately into a half volume by exposing to dry air for three minutes to separate clearly into two layers. Thereby the major part of chlorophyll dissolved in petroleum ether, and float. The extract were concentrated approximately into a half volume by exposing to dry air.

2.2. High Isolation and Characterization of Phenolic Compounds by Performance Liquid Chromatography (HPLC)

The prepared phenolic extracts were used to determine the phenols quality of each species by using HPLC, the analysis was

carried out at National Center of Supervision and Medicinal Research – Ministry of Health-Baghdad.

HPLC Conditions was; analytical column: eurospher 100, C18, 5µm, 250 x 4.6 mm.; mobile phase: acetonitrile: water: concentrated phosphoric acid (400: 600: 5); flow rate: 0.8 ml/in.; detection wave length: 254nm; temperature: 25 °C.

2.3. Thin Layer Chromatography (TLC)

TLC was used as another method for phenolic compounds detection. Germany plate silica gel with dimensions 20x20cm was used, after activation in oven at 100-110 C^{O} for 20-30min was used. Small spots of prepared phenolic extracts were added on silica plate (lower part) by capillary tubes with leaving 2cm distance empty on all sides of plate (upper, lower and both lateral sides). Thus, spots of each standard phenolic compound were added beside the spot of extraction, with omitting 2cm distance among each one. Spotting were repeated several times to saturated and left to dry. Plates with spots placed in a suitable developing glass tank, which contains solvent then covered. The solvent was prepared and put in the tank before placing the plates even to make solvent steam and filling the tank. Four different solvents were used to isolate and study phenols [9], included; Forestall (Acetic acid: Concentrated HCl: Water; 30:3:1 0); BAW (n-Butanol: Acetic acid: Water: 4:1:5 top layer); HoAc 50% (50% aqueous acetic acid); 10% Acetic acid in chloroform. The BAW is the best for the separation of phenolic compounds on silica gel plate which was prepared by mixed n-butanol 80ml, Glacial acetic acid 20ml and distilled water 100ml in separating funnel and shacked gently then left even to separate into two layers. The upper layer was taken and used in next steps. Plates left in the tank for 8-9 hours, whereas the solvent move (traveled) to specific distance (16cm) for each specimen. Plates brought out and dried. Plates were treated with developer such as ammonia vapor, by thereby the tank space was saturated by ammonia vapor putting small opened tube containing ammonia solution inside the tank, then covered and left for short time. Thereafter, the plates were placed inside the tank for 20-30 minutes, and were taken out the plates and recorded notes. Plates tested under UV ray (365nm), and observations were recorded. Phenolic compounds were recognized by retardation factor (relation flow Rf), color features in normal light and in UV ray. This was done by comparing Rf of colors for specimens and Rf of standards. Separation and identification of phenolic compounds was made randomly by placing one specimen with number of standards on one plate.

3. Results and Discussions

his study conducted in two ways, pure standards were used with HPLC method and impure standards were used with (TLC). The results by HPLC were 14 phenolic compounds in nine different species, (1-9) and by TLC 5 compounds (10-14) (Table 1).

Based on the number of compounds, the tested species can be divided into: species contain 2 phenolic compounds such as *G. molle* (6,14); species with 3 compounds as in *G. dissectum* (1,7,11), *G. pusillum* (4,5,14), *G. stepporum* (5,11,13) and *G. tuberosum* (7,10,13); species contain 4 compounds as in *G. purpureum* (1,2,13,14)*;* species with 5 compounds as in *G. divaricatum* (1,3,4,13 and 14), *G. kurdicum* (1,5,6,12 and 13), *G. lucidum* (1,5,7,12,14) and *G. rotundifolium* (5,6,8,11 and 13). The compounds (4-allyleanizol and quercetine) were used and they gave retention time and relation flow of HPLC and TLC. However, they had not been found in the studied species. Compound 2, 3, 9 and 11were recorded lowest distribution (9%) each distributed as a single species. Compounds 8 and 11 were recorded distribution 18% each compound was found in 2 species. Compounds 4, 6 and 7, 14 with 27% distribution each compound was found in 3 species. Compounds 5 and 13 with 45.4% distribution each was found in 5 species and the compounds 1 and 12 with highest distribution 54.5% each compound was found in 6 species. Similar species also can be distinguished by a difference in the number and quality of phenolic compounds they contain. For example, species *G. molle* and *G. pusillum* which contain the same compound they are similar in morphology. However, *G. molle* contains compounds 6 and 13 and *G. pusillum* with 4, 5 and 14. Also, *G. stepporum* contain compounds 5, 10 and *G. tuberosum* contain 7, 9 despite their similarity. On the other hand, the compound 7 was found in dissimilar species *G. dissectum, G. lucidum* and *G. tuberosum* and compound 4 was found in *G. divaricatum* and *G. stepporum.* Accordingly, the quantity, quality and concentration of phenolic compounds can be used to distinguish and separation species similar in morphology

Table 1: Phenolic compounds in the studied species

No.	Phenol compound	Found in
1	Solver Ethanol	*G. dissectum, G. kurdicum, G. divaricatum, G. lucidum* and *G. purpureum.*
2	Caffeine	*G. purpureum*
3	Coumarin	*G. divaricatum*
4	Vanillin	*G. divaricatum* and *G. pusillum*
5	4-Methyl Phenol	*G. kurdicum, G. lucidum, G. pusillum, G. rotundifolium* and *G. stepporum*
6	2-6 Dimethyl Phenol	*G. kurdicum, G. molle* and *G. rotundifolium*
7	Salicylic acid	*G. dissectum, G. lucidum* and *G. tuberosum.*
8	P-Cresol	*G. rotundifolium*
9	Eugenol	*G. tuberosum*
10	Alizarin	*G. dissectum* and *G. rotundifolium*
11	Catechin	*G. rotundifolium*
12	Chloroginic acid	*G. dissectum, G. kurdicum, G. lucidum, G. purpureum* and *G. stepporum*
13	Gallic acid	*G. divaricatum, G. molle, G. purpureum* and *G. rotundifolium.*
14	Syringic acid	*G. divaricatum, G. lucidum* and *G. pusillum*

4. Conclusions

This study concluded that there were different species (non-similar morphologically) which contain the same phenolic compounds and there were phenolic compounds which occurred in the same species. The reason for the differences can be due to genetic or / and environmental factors. Therefore, further studies are needed in this area.

Supplementary Information

Supplementary information related to this article can be found at: (Supplementary) Salih, S. H. Passer 1 (2019) 01- 03

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