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Qualitative and Quantitative Estimation of Total Phenols in *Narcissus tazetta* L. Bulbs

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Abstract

Narcissus tazetta, a member of the Amaryllidaceae family, is known to be rich in bioactive metabolites such as alkaloids, phenolics, and flavonoids, which have been found in nearly every species in this family. *N. tazetta*, cultivated in Iraq, had not previously been studied for its active components; thus, the current study used phytochemical screening and phenolic compound estimation, both qualitatively and quantitatively. Results showed that the plant alcoholic extract was rich in alkaloids, polyphenols, and flavonoids, as well as tannins, polysaccharides, and saponins. Qualitatively, TLC and HPLC chromatograms for total phenolics and flavonoids compounds revealed the presence of gallic acid (GA), caffeic acid (CA), paracumarin acid (P-c), chlorogenic acid (Ch), catechin (Ca), and hydroqunolinon (H) with more abundant content in vanillic and salicylic acids as phenolic compounds; besides, rutin (R), quercetin (Q), apigenin (Ap), Luetolin (L), and kaempferol (K) as flavonoids. Dried bulbs of *N. tazetta* contain total phenolic compounds of 90 mg/g and total flavonoids of 0.47 mg/g. Plant bulb analyzed by colorimetric reaction assay.

Keywords: Amaryllidaceae family, *Narcissus tazetta*, Total flavonoids, Total phenols.

1. Introduction

The plant *Narcissus tazetta* belongs to the family Amaryllidaceae, which is known worldwide for its use in folk medicine and as an ornamental in gardens and parks due to its beautiful

flowers. About 1600 species are grouped under this family and divided into 85 genera distributed throughout tropical and subtropical regions of the world [1, 2]. Plants of this species are widely distributed throughout the Mediterranean regions, from Portugal to Turkey; they are also found in the Middle East, Central Asia, Australia, Mexico, and the USA. Flowers bloom in early spring [3]. The *Narcissus* genus is one of the most important spring-flowering plants. A short botanical description of phytochemically investigated Narcissus species is summarized in the following sections: *Narcissus pseudonarcissus, Narcissus poeticus, Narcissus jonquilla, Narcissus serotinus, Narcissus serotinus, Narcissus triandrus, Narcissus assouanus, Narcissus. Bujei, Narcissus confuses,* and *Narcissus tazetta*. The chemical analysis of *Narcissus tazetta* is rich with biologically active components, among them cardioactive glycosides, different types of flavonoids, alkaloids, tannins, cardiac glycosides, volatile oil, steroids, terpenoids, and anthraquinones [4, 5]. All these components might give the plant a unique importance as an antifungal [6], antiviral [7], anticancer [8], antioxidant [9], in the treatment of cardiovascular disorders [10], immune-boosting agent [11], in cases of malaria disorders, and recently as acetylcholine esterase inhibitory effects to treat Alzheimer's conditions [12]. The plant is rich in phenolic compounds, which are the most explored group in nature and have expressed potential for health benefits [13]. Generic terms like ‘phenolic compounds’, 'phenolics, or ‘polyphenolics’ refer to more than 8,000 compounds found in the plant kingdom that possess at least an aromatic ring with one or more hydroxyl substituents, including functional derivatives like esters, methyl ethers, glycosides, etc. [14]. Phenolic compounds found in food materials are divided into two groups: simple phenols and polyphenolic compounds; flavonoids represent the major group of phenolics [15]. These secondary metabolites are produced in almost all plant kingdoms via the shikimic acid pathway [16]. The antioxidant properties of these compounds have a role in the prevention of many diseases, such as chronic cardiovascular diseases, cancer, and neurodegenerative diseases. In spite of their wide distribution in the plant kingdom, researchers have focused their attention on the health benefits of phenols [13]. Given the importance of phenolic compounds, the current study was used to investigate the phytochemicals present in bulbs of *Narcissus tazetta* cultivated in Iraq and to estimate the quantity and quality of phenolic compounds in this part of the plant.

2. Materials and methods

2.1. Plant collection

The bulbs of *Narcissus tazetta* were obtained from plants grown in the gardens of the Zyounah area of Baghdad, Iraq. Scaly tips and leaves were removed, and the bulbs were washed thoroughly with water and prepared for subsequent experiments.

2.2. Plant total phenolic compounds Extraction

About 350g of fresh small pieces from cleaned bulbs were subjected to 750 ml of n-hexane to remove any fat materials from the sample. The weight of the cut bulbs after fat removal and drying was 140 g; which was macerated with 1000 ml of 80% ethanol alcohol and left for a week in the dark with stirring. The sample was filtered and finely dried using a rotary evaporator at a temperature of 45°C. This process was repeated to extract most of the active compounds in the plant [17].

2.3. Detection of the active compounds of the ethanolic extract of Narcissus bulbs

The following tests were applied to detect the major active component in the alcoholic extract at a concentration of 0.5 mg/ml:-

2.3.1. Detection of Alkaloids (Dragendorff test)

Two solutions were freshly prepared for this test: Weigh the Bismuth subnitrate solution of 60 mg/0.2 ml Hydrochloric Acid as solution A and the potassium iodide Solution 600 mg/1 ml distil water as solution B. The fresh mixture solution [A + B] will give an orange-brown precipitate as the plant extract has been added [19]. 2. Detection of polysaccharides Benedict reagent was pursued from stores of lab reagents. The appearance of a reddish deposit at the addition of Benedict reagent to 1 ml of the plant extract placed in a boiling bath will indicate the presence of this group.

2.3.2. Detection of Tannins

In this test, the plant extract was subjected to a few drops of tannin's reagent, composed of a 1% lead acetate solution. Positive results showed a white or gelatinous precipitate.

2.3.3. Detection of Saponins

When an aqueous or water-alcoholic plant extract has been shaken vigorously for a few seconds, the foam formation that persists for minutes will indicate the presence of Saponins.

2.3.4. Detection of Flavonoids

For detection of the flavonoid content in the plant extract, a test called the Alkaline test has been applied: by adding drops of NaOH solution to the plant extract, a bright yellow color will appear.

2.3.5. Detection of Polyphenolic compounds

The plant extract will show dark brown deposition as a few drops of 3% ferric chloride solution have been added [18].

2.4. Qualitative Determination of Phenols Using Thin Layer Chromatography (TLC)

The Narcissus total phenol (Tp) extract solution was prepared at a concentration of 0.5 mg/ml with absolute ethanol. Standards flavonoids and phenolic compounds that were prepared with ethanol included:R=Rutin, Q=Quercetin, Qr=Qurecetrine, Ap=Apegenin, L=Luetolin, K=Kaempferol, GA=Gallic acid, CA=Caffeic acid, Py=Pyrogallol, P-c=paracumaricacid, Ch=chlorogenic acid, Ca=Catechin, H=Hydroqunolin.

Thin-layer chromatography (TLC) was performed using an aluminium plate covered with a thin layer of silica 60 as the stationary phase. As for the mobile phase, Ethyl acetate: Formic acid (9:1) was used. The distance travelled by each separated compound is called the relative flow (Rf) value.

$$Rf = \frac{The\ distance\ travelled\ by\ each\ model}{The\ distance\ travelled\ by\ the\ mobile\ phase}$$ [20]

2.5. Quantification Determination of total phenols in the plant extract

The detection was done with the Folin-Ciocalteau reagent. An aliquot of 1 ml of the plant ethanolic extract was taken at a concentration of 10 mg/10 ml D.W. and placed in a glass tube to become the final concentration of the extract (1 mg/ml), and then 1 ml of Folin-Ciocalteau reagent was added. After 5 minutes, 10 ml of sodium carbonate (Na_2CO_3) solution at a concentration of 7 g/100 ml was added to the tube and mixed well with a vortex, then 13 ml of distilled water was added. The mixture was left in a dark place for one hour, and the absorption was measured at a wavelength of 760 nm by a UV Spectrophotometer.

The total amount of phenols in the plant extract was calculated by making a standard curve using (Gallic acid) as standard phenol with concentrations of (30, 40, 60, 80 and 100 µg/ml), and performing the same reaction steps above, and measuring the absorbance of each concentration, and based on the graphic relationship between acid concentration and absorption at a wavelength of 760 nm, the straight line equation was extracted to calculate the total amount of phenols for Narcissus plant [21].

2.6. Quantification Determination of total Flavonoids in the plant extract

The total flavonoid content of plant extract was determined by preparing Rutin standard flavonoids in serial concentrations ranging from (0.156 to 2.5) mg/ml. The extract concentration was 200 mg in 100 ml of (50%) ethanol. A colour reaction was obtained by treating 1 ml of each Rutin standard and the extract with 1 ml of (5%) sodium nitrite solution and leaving them at room temperature for 5 minutes. An aliquot of 2 ml of (10%) aluminum chloride was added to all tubes to be left for another 5 minutes at 250 C. At last, all tubes were read at 510 nm after adding 5 ml of 1N NaOH solution. The plotted standard curve between the absorbance of standard solutions against their concentrations and the straight line equation was used for calculating the concentration of extracted flavonoids in plants [22].

2.7. Qualification and Quantification of total phenols and total flavonoids in the plant

For total phenolic compounds investigation the following HPLC conditions were applied [23]:

Column: ODS L18 (10X 4.6Id)mm, 5µm particle size

Flow Rate: 0.7ml/min.

Injected volume: 20 µl

Detector: UV - Vis.

Wave length: 280nm

Mobile Phase: A=1% Acetic acid and B=Methanol 10%

The plant extract concentration was 12.5mg/ml methanol

For total Flavonoids investigation the following HPLC conditions were applied:

Column: ODS L18 (5.0X 4.6Id) mm, 3µm particle size

Flow Rate: 0.5 ml/min.

Injected volume: 20 µl at room temp.

Detector: UV - Vis.

Wave length: 338nm

Mobile Phase: A=0.5% formic acid and B=Acetonitrile 80%

The plant extract concentration was 12.5mg/ml methanol

3. Results

The fresh small pieces in the 350 g bulb yielded a defatting residue of 140 g, which means about half the bulb weight is fatty material. The defatted residue that was macerated later with 1000 ml of 80% ethanol gave a yield of 0.9544g (**Table 1**).

Table 1. Weight of *N. tazetta* bulbs before and after defatting and ethanolic extraction.

Plant	Weight of bulbs before defatting (g)	Weight of bulbs after defatting (g)	Dried n-Hexane layer (mg)	Dried 80% Ethanolic extract residue (g)
Narcissus tazetta	350	140	120	0.9544

3.1. Detection of the active compounds of the ethanolic extract of Narcissus bulbs

The results of chemical analyses and detections of the crude alcoholic extract of Narcissus bulbs indicated that the plant was rich in many bioactive molecules such as alkaloids, phenols, flavonoids, saponines, and others.

Table 2. The main active compounds in the alcoholic extract of Narcissus bulbs.

Test	comments	Results
Detection of Polyphenolics	Brown p.p.t.	+
Detection of Flavonoids	Bright yellow	+
Detection of Tannins	White p.p.t.	+
Detection of polysaccharides	Orange-Red p.p.t.	+
Detection of alkaloids	brown p.p.t.	+
Detection of the Saponins	Foam formation	+

***Results (+) represent the presence of these compounds in the plant**

3.2. Qualitative Determination of Phenols Using Thin Layer Chromatography (TLC)

Results for phenols and flavonoids qualitatively assayed by TLC technique estimated in the plant bulb revealed the presence of several compounds like Rutin, Quercetin, Quarcetrin, Apegenin, Luetolin, Kaempferol, Total phenols, Gallic acid, Caffeic acid, Pyrogallol, paracumarin acid, chlorogenic acid, Catechin and Hydroqunolin. **Figure 1.** The detected phenols

and flavonoids with their Rf values in comparison with standard values are represented in **Table 3**.

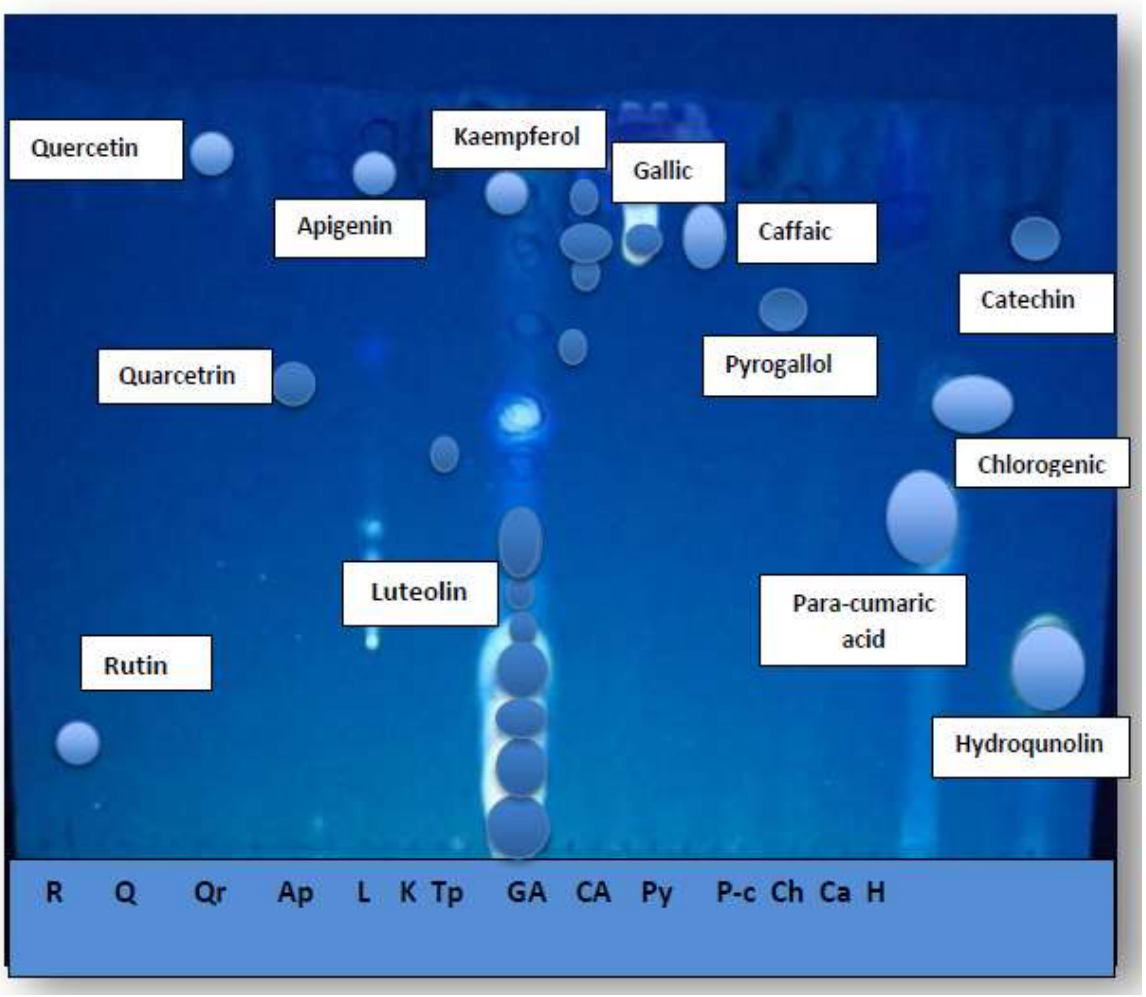

Figure 1. TLC chromatogram for *N.tazeeta* total phenolic and flavonoids compounds; R=Rutin, Q=Quercetin, Qr=Quarcetrin, Ap=Apegenin, L=Luetolin, K=Kaempferol, Tp=Total phenols, GA=Gallic acid, CA=Caffeic acid, Py=Pyrogallol, P-c=paracumarinacid,Ch=chlorogenic acid, Ca=Catechin, H=Hydroqunolin.

Table 3. The Rf Values of the standards and extracted phenols and flavonoids.

	Rf Values												
Compound	**R**	**Q**	**Qr**	**Ap**	**L**	**K**	**GA**	**CA**	**Py**	**P-c**	**Ch**	**Ca**	**H**
Rf Standard	0.143	0.89	0.63	0.89	0.85	0.92	0.82	0.78	0.835	0.8	o.6 0.46	0.79	**0.28**
Rf Extracted phenols (Tp)	**All the Above spots and others**												

3.3. Quantification Determination of total phenols in the plant extract

The amount of phenols present in the common Narcissus plant was estimated based on the standard phenol Gallic acid using a spectrophotometer at the wavelength of 760 nm and based on different concentrations of Gallic acid solution (**Table 4)**.

Table 4. Absorbance values for several concentrations of standard phenol Gallic Acid.

Gallic Acid concentration (µg/ml)	Absorbance values (760 nm)
0	0
30	0.17
40	0.19
60	0.338
80	0.408
100	1.416
Total phenols of the plant extract	0.522

According to the absorbance value of Gallic acid in **Table 4,** the equation of the straight line was obtained from drawing the standard curve of the standard phenolic compounds in **Figure 2.**

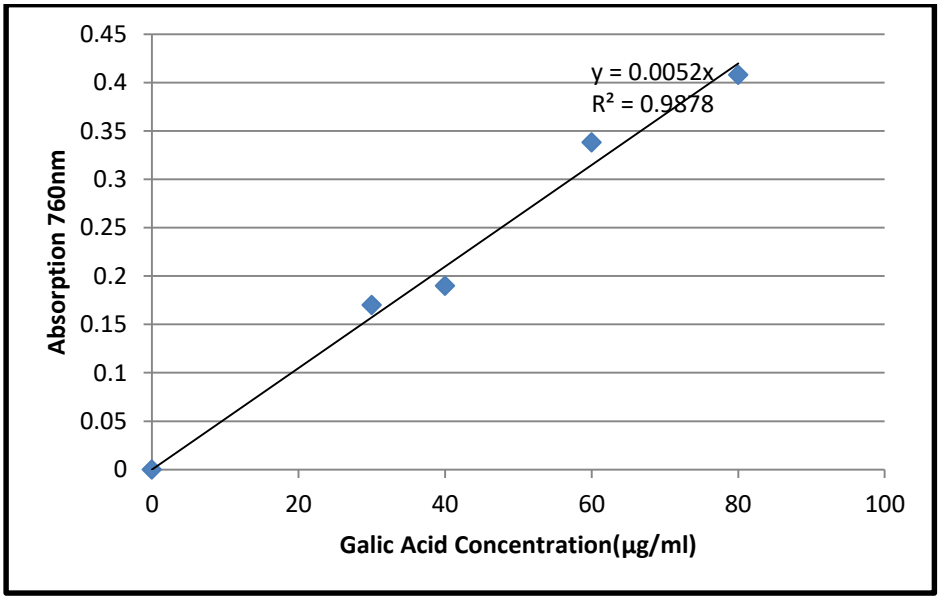

Figure 2. Standard Curve for Standard Phenol Gallic acid.

Then the results of the total phenol concentration of the plant extract were obtained from the equation of the straight line of the standard curve for the standard phenol Gallic Acid with different concentrations as follow:

Y=0.0052X Y=Absorbance, X=Total phenol concentration (µg/ml)

X=0.522 / 0.0052= 100.4 µg/ml

Each 140 g bulb contained a total phenol of 96.2 mg

3.4. Quantification Determination of total Flavonoids in the plant extract

The average reading of different Rutin standards of absorption for each concentration is shown in Table 5. The plotted curve represented by Rutin standard concentration (X axis) against their absorption at 510 nm (Y axis) with the equation of a straight line is shown in **Figure 3**.

Table 5. Absorption reading of standard Rutin against each concentration and total flavonoids of plant extract.

Rutin Standard (mg/ml)	Average reading at 510 nm
0.15625	0.135
0.3125	0.341
0.625	0.602
1.25	1.003
2.5	2.750
Total flavonoids of the plant extract	0.111

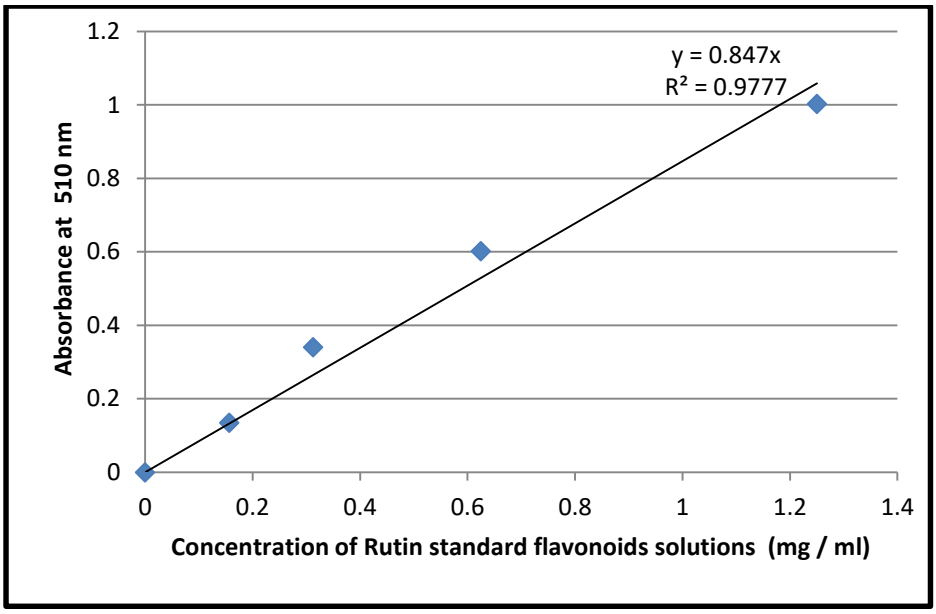

Figure 3. Standard Curve for Standard flavonoids Rutin.

After application of the straight line equation, **Figure 3,** the total flavonoids in bulbs could be calculated as shown:

X = 0.111 / 0.847

X=0.131 mg/ml total flavonoids in each 2 mg residue

Each 140 g bulb will contain 62.5 mg total flavonoids

That means each 1g weight of Narcissus bulbs should contain 0.47 mg total flavonoids as rutin.

3.5. Qualification and Quantitative determination of total phenols and Total Flavonoids by HPLC

3.5.1. Total Phenol

Figures 4 and 5 investigated the major phenolic compounds in the standard and sample extracted solution respectively.

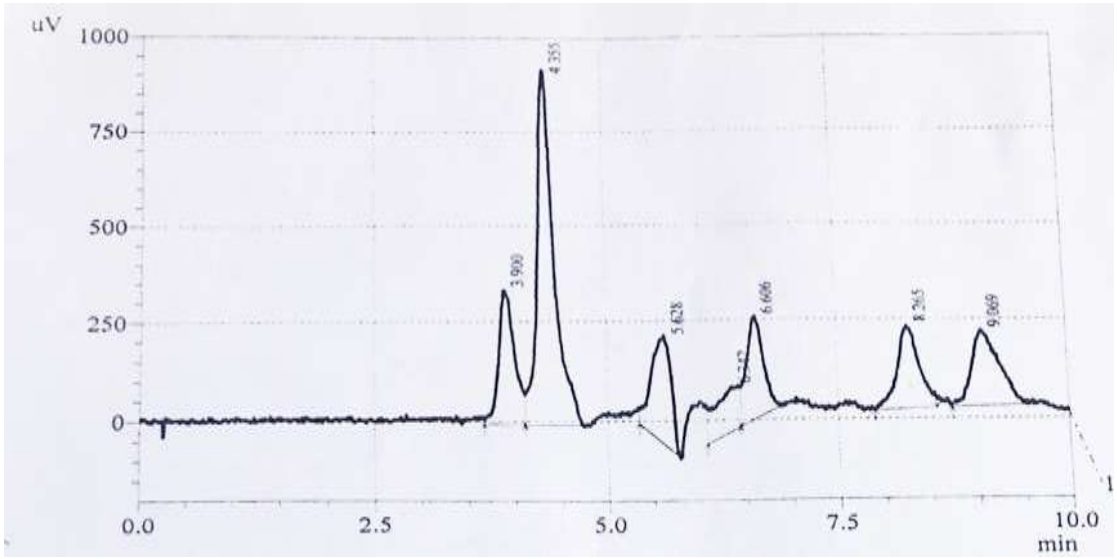

Figure 4. HPLC chromatogram for standard phenolic compounds

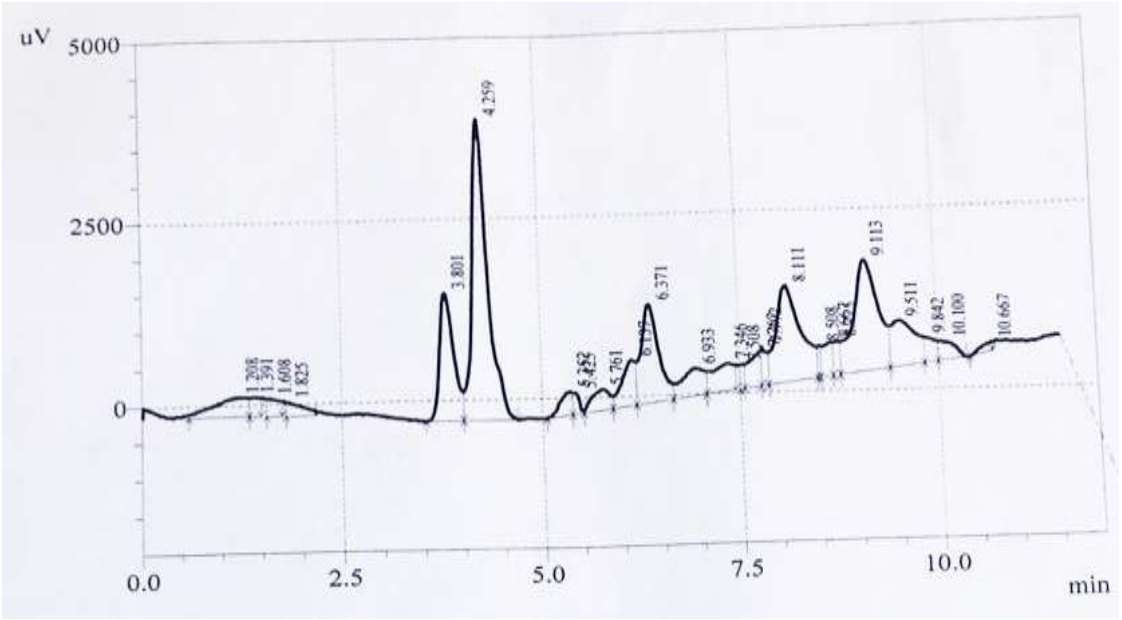

Figure 5. HPLC chromatogram for plant extracted phenolic compounds in Narcissus bulbs.

Chromatographic analysis of bulb extract revealed the presence of 6 phenolic compounds in different concentrations **Table 6.** These were vanillic acid, Caffeic acid, p-coumaric acid, Chlorogenic acid, Sinapic acid and Salicylic acid. The proportions of these phenolic compounds varied between (4.64 - 4.82 µg/g) in Sinapic acid and Salicylic acid respectively, and 0.70 µg/g in p-Coumaric acid.

Table 6. HPLC analysis results for standards and the extracted phenolic compounds.

Phenolic compound	Conc. µg / ml	Rt.in minutes For Standarad phenols	Area under the curve For Standarad phenols	Rt.in minutes For the extracted phenols	Area under the curve For extracted phenols	Concentration µg / ml	Concentration µg /g.plant
Vanillic acid	1	3.900	4065	3.801	21312	5.24	3.00
Caffeic acid	1	4.355	1148	4.259	55293	4.80	2.75
P-coumaric acid	1	5.628	3928	5.761	46470	1.20	0.70
Chlorogenic acid	1	6.606	3311	6.933	76060	2.30	1.30
Sinapic acid	1	8.265	3548	8.111	28843	8.12	4.64
Salycilic acid	1	9.069	4005	9.113	33813	8.44	4.82

3.5.2. Total Flavonoids

Figures 6 and 7, investigated the main flavonoids compounds in the standard and sample extracted solution respectively.

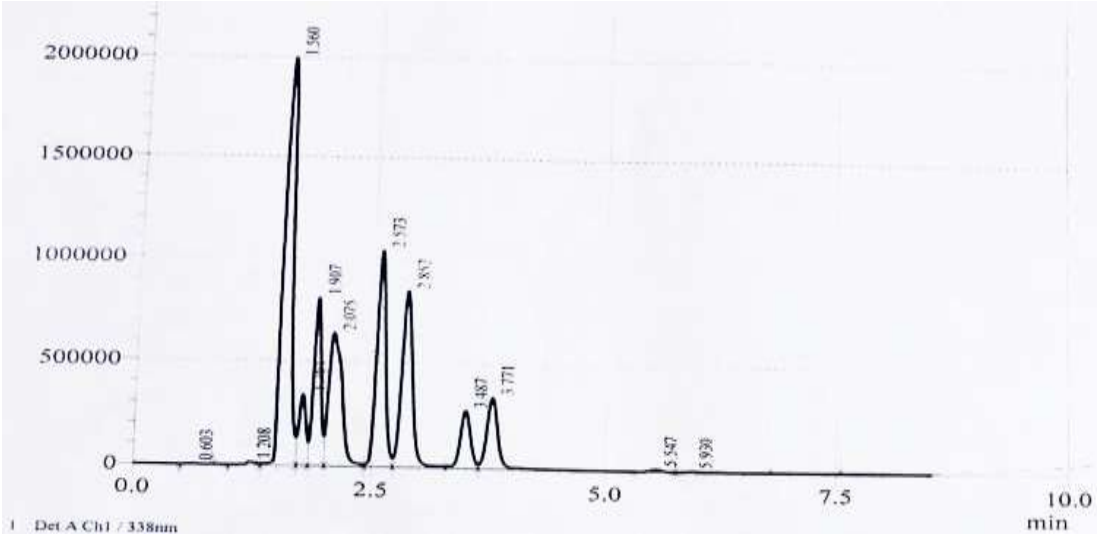

Figure 6. HPLC chromatogram for standard flavonoids compounds.

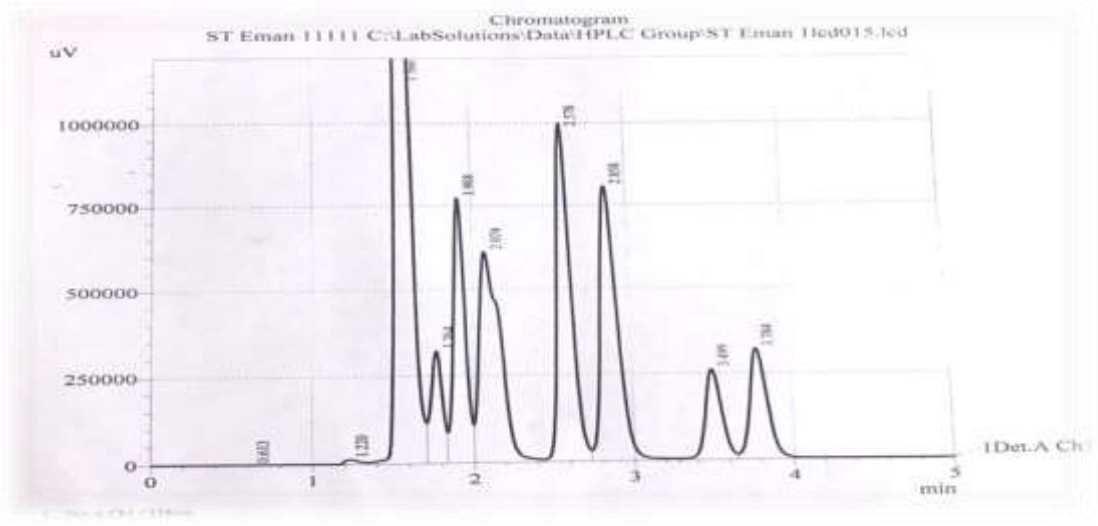

Figure 7. HPLC chromatogram for plant extracted flavonoid compounds in Narcissus bulbs.

Chromatographic analysis revealed the presence of eight flavonoid compounds **Table 7**. Rutin, Quercetin, Apigenin, Naringenin, Catechin, Luteolin, Commarin and Kaempferol were identified in the alcoholic extract of Narcissus bulbs. The Highest concentration of flavonoids was scored by Rutin (2.64 µg /g), and less concentration was scored by Kaempferol (2.43 µg /g). All detected flavonoids are in a close range.

Table 7. HPLC analysis results for standards and the extracted flavonoid compounds.

Flavonoid compounds	Conc. µg / ml	Rt.in minutes For Standarad flavonoid	Area under the curve For Standarad flavonoid	Rt.in minutes For the extracted flavonoid	Area under the curve For extracted flavonoid	Concentration µg / ml	Concentration µg /g.plant
Rutin	5	1.560	13932030	1.560	12871326	4.62	2.64
Quercetin	5	1.764	1752856	1.764	1575807	4.50	2.57
Apigenin	5	1.907	4361168	1.908	3919253	4.50	2.57
Naringenin	5	2.075	6163333	2.074	5563256	4.51	2.58
Catechin	5	2.573	6794463	2.578	6144692	4.52	2.58
Luteolin	5	2.852	6685820	2.858	6001127	4.49	2.57
Commarin	5	3.487	2098573	3.499	1864912	4.44	2.54
Kaempferol	5	3.771	2959933	3.784	2517318	4.25	2.43

4. Discussion

Active components of plants have an important role in diminishing and protecting a wide range of human abnormalities, such as anticancer, immune enhancement, blood and cardiovascular disorders, and others. All this medical importance may indicate their effective mechanism as free radical scavengers with antioxidant activity, especially the flavonoids and different phenolic compounds [24]. Flavonoids, phenolic compounds, and essential oils are important for maintaining good health in humans [25].

In this study, phytochemical analysis showed, as may be expected, the presence of many bioactive compounds in the bulbs of *Narcissus tazetta*, like Alkaloids, phenolics, flavonoids, saponines, tannins, and cardiac glycosides. These results are confirmed by other studies [26].

The bulbs of *Narcissus tazetta* are rich in different biologically active components, as chemical analysis revealed, and these results were approved by other studies [27]. It was shown that 80% ethanolic solution was a good solvent for total phenolic extraction and yielded an amount greater than the total flavonoid content in bulbs. The total phenols of the plant extract were 0.522, while the total flavonoids of the plant extract were 0.111.

Focusing on the phenolic and flavonoid chemicals in *Narcissus* bulbs, many of them are likely and expected to be discovered. By using HPLC analysis, six phenolic compounds and eight flavonoid compounds were identified in various quantities. The largest phenolic chemicals in bulbs were salicylic, sinapic, and vanillic acids, whereas all flavonoid components were present in similar amounts, with rutin being the most abundant. *Narcissus* bulbs were high in phenolics. In comparison to flavonoid compounds (62.5 mg/140 g bulb), total phenolic compounds have a higher concentration (96.2 mg/140 g bulb).

In their study on *Narcissus tazetta* flowers, [28] found that flavonoids were more prevalent than phenolic components; for every 5 kg of the ethanolic extract, total phenolic was 64.14

mg/ml, while flavonoids were 70.79 mg/ml. In the *Narcissus tazetta* flower, [29] discovered substantial concentrations of total polyphenols and flavonoids. They confirmed that the Amaryllidaceae family is able to synthesize a diverse range of polyphenols in addition to alkaloids. These bioactive molecules are well-known for their antioxidant activities [30, 31].

The type and amount of phenolic and flavonoid chemicals in Narcissus plants depend on many factors, like plant part, season, genotype, and methods of extraction. All these results gave the plant scientific validation for its biological activity, as in folk usage and even in recent medicine [17].

5. Conclusion

Narcissus tazetta bulbs of the Iraqi cultivar are rich in phenolic and flavonoid compounds, in addition to the presence of alkaloids, tannins, polysaccharides, and Saponins. The concentration of phenols was higher than that of flavonoids. These compounds gave the plant its scientific validation as a good source of huge compounds responsible for its biological activity in folk usage and even in recent medicine. More studies will be needed on wild species grown in Iraqi mountains to compare their phytochemical compounds with those of cultivated species.

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