Rumex conglomeratus Murr. Grown Wild in Syria: Phytochemical Analysis and In Vitro Antioxidant Activities of Aerial Parts and Rhizomes Extracts

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ABSTRACT

Rumex conglomeratus Murr. (Polygonaceae), has been traditionally used to treat various conditions including skin-ailments, infections, constipation, and cancer. The medicinal importance of *Rumex* plants stems from their richness in many bioactive secondary metabolites. This study represents the first report on the chemical constituents and antioxidant activity of *Rumex conglomeratus* aerial parts and rhizomes extracts. The aqueous and ethanolic extracts were prepared and preliminary phytochemical screening tests were conducted. Total phenols, flavonoids, and anthraquinones contents were determined, along with the antioxidant activities, using colorimetric methods and a UV-visible spectrophotometer. The results revealed that *R. conglomeratus* is a rich source of secondary metabolites. Rhizomes ethanolic extract showed the highest content of phenols (502.55 ± 1.36 mg GAE/g DE) and anthraquinones (6.71 ± 0.106 mg RhE/g DE). It also exhibited the highest antioxidant activity as DPPH free radical scavengers (IC50 = 5.40 ± 0.380 mg/L), and as reducing agents in the FRAP assay (0.230 ± 0.004 at 200 mg/L), and TAC assay (321.41 ± 6.94 mg AAE/g DE). These findings suggest the potential use of *R. conglomeratus* as a potent antioxidant or even as a laxative agent. However, further research is essential to confirm the safety and efficacy, emphasizing the importance of continued exploration to isolate and identify the biologically active compounds.

Keywords: *Rumex Conglomeratus*; Polygonaceae; Phenols; Anthraquinones; Flavonoids; Antioxidant

1. INTRODUCTION

Oxidative stress has been involved in the pathophysiology of many life-threatening diseases, including cancer, cardiovascular diseases, neurodegenerative disorders, atherosclerosis, inflammation, and aging. Oxidative stress is a physiological condition that occurs when there is an imbalance between the body's antioxidant defense system and the production of free radicals, which are highly reactive atoms or molecules that have one or more unpaired electron[1,2]. The accumulation of free radicals, primarily reactive oxygen species (ROS) and reactive nitrogen species (RNS), can damage cellular structures such as proteins, lipids, and DNA, leading to oxidative damage in the body. This could occur under specific conditions, such as exposure to environmental toxins, chronic inflammation, or a poor diet[3].

Antioxidants are compounds that can neutralize free radicals and protect cells from oxidative damage. In recent years, there has been a significant attention on natural antioxidants derived from medicinal plants due to their safety and health benefits[4], and their high production of natural antioxidants such as vitamins C and E, carotenoids, and polyphenols[5,6].

Polyphenols, including phenolic acids, flavonoids, anthraquinones, tannins, stilbenes, and lignans, are the most significant class of secondary metabolites, exhibiting potent antioxidant activities through various mechanisms[7,4,8]. Anthraquinones, particularly phenolic-

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substituted anthraquinones, are abundant in the Polygonaceae plants, including *Rumex* L. species, and can inhibit the formation of free radicals or scavenge them through direct or indirect mechanisms[9,1].

The *Rumex* L. genus (Polygonaceae) is known for being rich in secondary metabolites, including anthraquinones, naphthalenes, flavonoids, tannins, stilbenes, terpenes, phenolic acids, and others[10]. The diverse chemical composition of *Rumex* L. species has made them a focal point for research and investigation of biological activities, including antioxidant, antibacterial, antiviral, antitumor, and antidiabetic properties[11,12]. *Rumex* L. plants have been utilized in traditional medicine to treat various health conditions such as skin ailments, bleeding, inflammation, constipation, and tumors[12,13]. Roots have been used as dyes due to their anthraquinones content. Leaves of many species are characterized by a sour taste, which is attributed to the presence of oxalic acid derivatives, and have been eaten as vegetables[12].

Rumex conglomeratus Murr., known as "clustered dock", and is called in Arabic "Hommaid or Hommaidah", is a wild edible plant spread widely in the northern temperate zone and Mediterranean region[14,15]. It has been used traditionally to treat scurvy, skin burns, rashes, eczema, and cancer. The leaves and aerial parts have been consumed as food[13,11].

To our knowledge, there are no previous studies on the chemical composition and biological activities of rhizomes and aerial parts of *R. conglomeratus*. Kilic *et al.* conducted a study on the aqueous extract of *R. conglomeratus* leaves collected from Turkey. They estimated the total phenols, flavonoids, carotenoids, and proline contents and evaluated the antioxidant activity[16]. Marelli *et al.* determined the total flavonoids content of 13 plants, including *R. conglomeratus*, and reported the presence of stigmasterol, ergostenol, and neophytadiene in the ethanolic extract of the leaves. They also evaluated the antioxidant activity[17]. Other studies have examined the antibacterial activity of ethanolic extracts from the stems, leaves, and roots, revealing that the roots exhibited the highest activity against *Staphylococcus aureus*[18], while the methanolic extract from the aerial parts showed the highest activity against *Moraxella catarrhalis*[19]. Therefore, this study aims to investigate the composition of secondary metabolites, total phenols, flavonoids, and anthraquinones content, and to evaluate the *In-vitro* antioxidant activity of aqueous and 95% ethanolic extracts prepared from the aerial parts and rhizomes of *R. conglomeratus* (Figure 1).

Figure 1: *Rumex conglomeratus* Murr. aerial parts and rhizome.

2. GENERAL EXPERIMENTAL:

2.1 Chemicals

All chemicals and reagents used in the study were of analytical grade. Ethanol absolute and Quercetin (from SIGMA-ALDRICH, Germany), Methanol Absolute and Ascorbic Acid (from Panreac, Spain), Gallic Acid, Sodium Carbonate anhydrous, Monosodium Phosphate, Disodium Phosphate, and Trisodium Phosphate (from AvonChem, United Kingdom), Folin-Ciocalteu reagent, Sulfuric Acid, and Magnesium Acetate (from Merck, Darmstadt, Germany), Sodium Acetate, Chloroform 99%, Aluminum Chloride, and Ferric Chloride (from Riedel-de Haen, Germany), Rhein (from MCE MedChemExpress USA), Concentrated Hydrochloric acid (from SHAMLAB, Syria), DPPH· 2,2-DiPhenyl-1-PicrylHydrazyl (from Tokyo Chemical Industry, Japan), Potassium Ferricyanide and Ammonium Molybdate (from Titan Biotech LTD, India) Trichloroacetic acid (from Scharlau, EU).

2.2 Plant material:

Plants of *Rumex conglomeratus* Murr. were collected in May 2022 from Ain Mneen in Damascus Countryside, Syria, at an altitude of 1200 m, with coordinates 33°38′32″N 36°17′52″E. The species was identified by Prof. Ahmad Jadouaa, Faculty of Agriculture, Aleppo University, based on the New Flora of Lebanon and Syria[14]. The muddy underground parts were rinsed with distilled water. The aerial parts and rhizomes were separated and dried in shade at room temperature for two weeks. Then, they were ground using an electric grinder to prepare the extracts.

2.3 Extraction:

Four crude extracts were prepared using Ultrasound-Assisted Extraction with two different solvents (distilled water and 95% ethanol) for each plant part. 20 grams of plant powder were extracted with 200 mL of solvent (distilled water or 95% ethanol) using Ultrasonic water bath cleaner (Skymen 040S 40kHz Industry Digital Heated Ultrasonic Cleaner, China) at a temperature of 55 – 60 °C for 30 minutes. Then, the extracts were filtered. The extraction process was repeated until the extract's color disappeared. All filtrates were collected and dried using a rotary evaporator (Zhengzhou Great Wall Rotary Evaporator R-1001-VN, China) under low pressure and 40°C for ethanolic extracts, and 50°C for aqueous extracts. The extraction yields were calculated using the following equation, then the four crude extracts were stored at 4°C for further analysis.

Extraction yield % = weight of the dried extract / weight of the dried powdered plant material x 100

2.4 Preliminary Phytochemical Screening:

Qualitative detection tests were done on each extract to investigate the presence of flavonoids, anthraquinones, tannins, coumarins, saponins, alkaloids, and cardiac glycosides.

2.4.1 Tests for flavonoids

Each extract was dissolved in 20% ethanol at 50°C for 3 to 5 minutes and then filtered.

Aluminum chloride test: 1 mL of aluminum chloride reagent was mixed with 1 mL of the extract. Flavonoids were identified by the blue or green fluorescence under UV light (365 nm)[20].

Shinoda test: 3 mL of the prepared extract were dried and then dissolved in 1 mL of absolute ethanol. Next, pieces of magnesium metal were added with a few drops of concentrated hydrochloric acid. The formation of a red, orange, or purple color indicates the presence of flavones or hydroxyflavones[21].

2.4.2 Tests for Anthraquinones[22]

Borntrager's test: Each extract was mixed with 1 mL of chloroform for 10 minutes then filtered. 2 mL of 10% NH3 solution were added slowly. In the presence of free anthraquinones, a red color appears in the upper ammonia layer.

Modified borntrager's test: the extract was put in 5 mL hydrochloric acid HCl 7% and 2.5 mL of 5% ferric chloride, and boiled under reflux for 15 minutes then filtered. The filtrate was shaken with 4 mL chloroform in a separating funnel and continued as in the borntrager's test. A red color will appear in the aqueous layer indicates the presence of anthraquinones glycosides.

2.4.3 Tests for tannins[23]

The extracts were dissolved in distilled water, and boiled for 5 minutes, and then filtered.

Ferric chloride test: A few drops of 10% ferric chloride were added to 1 mL of the filtrate. The formation of a dark green or dark blue color indicates the presence of tannins or polyphenols.

Gelatin precipitation test (specific): 5 mL of filtrate were mixed with 1 mL of diluted acetic acid, and a few drops of 1% gelatin solution with 10% sodium chloride were added. A white-abrownish precipitate will appear in the presence of tannins.

2.4.4 Tests for coumarins[24]

Fluorescence test: The extracts were boiled with distilled water for 3 minutes and then filtered. The filtrate was exposed to 365 nm UV light. A blue fluorescence indicates the presence of coumarins.

2.4.5 Tests for Saponins[22,25]

Foam test: Each extract was put in a test tube with 10 mL of hot water for a few minutes, then shaken vigorously for about 20 seconds. The formation of stable foam for at least 10 minutes indicates the presence of saponins.

2.4.6 Tests for alkaloids[23]

Precipitation tests: Each extract was dissolved in 3 mL of 10% HCl and 15 mL of water. The mixture was heated for 5 minutes and then filtered. A few drops of Mayer, Dragendorff, Wagner, and Hager reagents were added to the filtrate. The formation of white, orange, reddish-brown, and yellow precipitates respectively indicates the presence of alkaloids.

2.4.7 Tests for cardiac glycosides[23]

The extracts were boiled with 15 mL of 50% ethanol and 5 mL of lead acetate solution under reflux for 10 minutes, then filtered and cooled. The filtrate was shaken in a separating funnel with 5 mL of chloroform, and this process was repeated 3 times, then the chloroform layers were collected.

Keller Kiliani's test: 5 mL of the prepared extract were dried and dissolved in 1 mL of glacial acetic acid. One drop of 5% FeCl3 and 1 mL of concentrated H2SO4 were added. The formation of a reddish-brown color at the junction of the two liquid layers, and the bluish-green color at the upper acetic acid layer indicate the presence of deoxysugars in cardiac glycosides.

Kedde's test: 2 mL of the chloroform extract were evaporated, followed by the addition of 2 mL of the reagent (2% 3,5-dinitrobenzoic acid in 90% alcohol) and 1 mL of an alkaline solution (20% sodium hydroxide solution). A purple color is produced in the presence of β-unsaturated-o-lactones (cardenolides).

2.5 Total Phenolic Content (TPC) in R. conglomeratus extracts:

2.5.1 Gallic acid standard and extracts preparation

A series of dilutions ranging from 0 to 500 mg/L was made from gallic acid standard solution in distilled water, and the extracts were prepared at a concentration of (600 mg/L).

2.5.2 Determination of total phenols

The total phenols content was determined using the Folin-Ciocalteu Reagent (FCR) method as described by Agha and Hussain[26], with slight modifications. Briefly, 40 µl of sample (Gallic acid or extract), 2 mL of distilled water, and 200 µl of 10% FCR were mixed. After 5 minutes, 600 µl of 10% Na2CO3 solution were added. Reaction tubes were covered, vortexed, and placed in the dark for 2 hours at room temperature. The absorbance reading of each sample was measured using a UV-visible spectrophotometer (Model: T80+, PG instrument Ltd, United Kingdom) at the maximum absorbance wavelength, λ_{max} = 762 nm. The blank was prepared with 2 mL of distilled water. The total phenolic content was calculated using linear equation of the calibration curve for Gallic acid ($y= 0.0012x + 0.0265$) ($R^2 = 0.9953$). All results were expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of the Dried Extract (DE).

2.6 ***Total Flavonoids content (TFC) in R. conglomeratus extracts:***

2.6.1 Quercetin standard and extracts preparation

A series of dilutions ranging from 0 to 50 mg/L was made from quercetin standard solution in methanol, and

the extracts were prepared at a concentration of 200 mg/L

2.6.2 Determination of total Flavonoids

The total flavonoids content was measured using the aluminum chloride colorimetric method described by Khatib and Al-Makky[27], with minor modifications. Briefly, 2 mL of the sample (quercetin or extract), 100 µl of 1 M sodium acetate, 100 µl of 10% methanolic aluminum chloride solution, and 2.8 mL of distilled water were mixed. Reaction tubes were covered, vortexed, and incubated in the dark at room temperature. The reaction will produce a yellow aluminum-flavonoid complex. After 30 minutes, the absorbance reading of each sample was measured using a UV-visible spectrophotometer at the maximum absorbance wavelength, λ_{max} **= 429 nm**. The blank was prepared with 2 mL of absolute methanol. The total flavonoids content was calculated using the calibration curve equation for quercetin **(y= 0.0274x + 0.0154) (R^2 = 0.9967)**. All results were expressed as milligrams of Quercetin Equivalents (QE) per 1 gram of the Dried Extract (DE).

2.7 ***Total Anthraquinones content (TAnC) in R. conglomeratus extracts:***

The total anthraquinones content was measured using the colorimetric method described by Sakulpanich and Gritsanapan[28]. The method depends on Borntrager's reaction principle, which is based on the ability of free anthraquinones (Aglycones) to react with alkali and form ions that are visibly pinkish-red colored[29].

2.7.1 Rhein standard

A series of seven dilutions ranging from 0 to 8.64 mg/L was made from Rhein standard solution in 0.5% magnesium acetate solution in methanol forming pinkish-red colors.

2.7.2 Extracts preparation

50 mg of the extract containing anthraquinones (Aglycones + glycosides) was accurately weighed, and 10 mL of distilled water were added. The mixture was stirred, weighed, and then refluxed on a water bath for 15 minutes. The flask was allowed to cool, weighed, and then adjusted to the original weight with distilled water. 20 mL of 10.5% FeCl3 and 1 mL of concentrated HCl were added (to break O-glycosides and C-glycosides bonds). The mixture was weighed and refluxed for 20 minutes, then allowed to cool and adjusted to the original weight, anthraquinones turned into the free aglycone form. The mixture was shaken with 25 mL of chloroform in a separating funnel, and the chloroform layer was collected, the extraction was repeated three times. The chloroform layers were filtered and transferred to a 100 mL volumetric flask, and the volume was adjusted with chloroform. 25 mL of the chloroform extract were evaporated and the residue was dissolved in 10 mL of 0.5% magnesium acetate in methanol (Alkali), resulting in a pinkish-red color.

2.7.3 Determination of total Anthraquinones:

The absorbance of the resulting pinkish-red color was measured using a UV-visible spectrophotometer at 517 nm. The total anthraquinones content (represents aglycones + glycosides forms) was calculated from the calibration curve equation for Rhein **(y= 0.0405x + 0.0038) (R^2 = 0.9976)**. All results were expressed as milligrams of Rhein Equivalents (RhE) per gram of the Dried Extract (DE).

2.8 ***In vitro antioxidant activity assessment of R. conglomeratus extracts:***

2.8.1 DPPH• free radicals scavenging activity:

The free radical scavenging activity of *R. conglomeratus* extracts was measured using the method of Kilic et al.[16], with some modifications. The extracts were prepared in five concentrations (5, 10, 25, 50, 100) mg/L from the aerial parts aqueous extract, and (2, 5, 10, 25, 50) mg/L from the other extracts. A series of six concentrations (0.5 to 10 mg/L) of gallic acid solution in ethanol was made. DPPH solution in ethanol (45 mg/L) was prepared. The reaction was done by adding 300 µl of the sample (gallic acid or extract) to 1500 µl of DPPH solution, and control was made by adding 300 µl of ethanol to 1500 µl of DPPH solution. The reaction was then incubated in the dark at room temperature. After 30 minutes, the absorbance was measured at 517 nm. The decreasing in absorbance indicates

greater radical scavenging activity. The percentage of DPPH free radical scavenging activity (RSA%) was calculated using the following equation, and the inhibitory concentration of 50% of DPPH free radicals (IC50 mg/L) was calculated for extracts and gallic acid from the graphs of the relationship between sample concentration and RSA%. The lowest IC50 value indicates the highest radical scavenging activity.

Radical Scavenging Activity % (RSA%) = 100 x (Ac – As) / Ac
Ac: the absorbance of control, As: the absorbance of sample

2.8.2. Ferric Reducing Antioxidant Power (FRAP Assay)

The reducing power of the *R. conglomeratus* extracts was determined according to the method described by Khatoon et al.[30], with slight modifications. Five different concentrations of extracts and ascorbic acid standard in methanol (25-200 mg/L) were prepared. 1 mL of the sample (extract or ascorbic acid), 2.5 mL of phosphate buffer (0.2 M, pH 6.6), and 2.5 mL of potassium ferricyanide (1 %) were mixed. The mixture was then incubated at 50ºC for 20 minutes. Next, 2.5 mL of 10 % trichloroacetic acid was added. After 10 minutes, the mixture was filtered. 1.8 mL of the filtrate were mixed with 1.8 mL of distilled water and 0.5 mL of 0.1% FeCl3. The absorbance was measured at **700 nm**. The increasing absorbance demonstrates the Fe^{+3} reducing power and antioxidant capacity of the sample. All measurements were done in triplicate and results were expressed as the mean ± standard deviation.

2.8.3. **Phosphomolybdate assay (Total Antioxidant Capacity TAC)**

The total antioxidant capacity was evaluated following the phosphomolybdate method described by Khatoon et al.[30], using ascorbic acid as a standard. Five different concentrations (25, 50, 100, 150, 200 mg/L) were prepared from extracts and ascorbic acid. 0.3 mL of the sample (extract or ascorbic acid) were mixed with 3 mL of the reagent (ammonium molybdate 0.4 mM, sulfuric acid 0.6 M, and trisodium phosphate 28 mM). The reaction tubes were incubated at 95°C for 90 minutes and then cooled to room temperature. In the presence of antioxidants, Mo (VI) is reduced to Mo (V) and forms a green-colored phosphomolybdenum (V) complex. The absorbance readings were measured at 695 nm. The TAC was calculated using the following equation of the ascorbic acid calibration curve ($y= 0.0043x + 0.1376$) ($R^2 = 0.9942$).

2.9 ***Statistical analysis:***

All measurements were done in triplicate. Results were expressed as the mean ± standard deviation. Correlation coefficient (r^2) between TPC, TFC, TAnC and the antioxidant activities were calculated using Microsoft Excel (2019) software

3. RESULTS

3.1 Yield of extraction:

The extraction resulted in four crude extracts, APA: Aerial parts aqueous extract, APE: Aerial parts ethanolic extract, RA: Rhizomes Aqueous extract, RE: Rhizomes Ethanolic extract. Table 1 shows the percentage yield of each prepared extract. The RA extract had the highest yield (28.5%), while the APE extract had the lowest yield (20.4%).

Table 1: Extraction yields of *R. conglomeratus* extracts*:*

Plant part	**Solvent of extraction**	**Yield%**
Aerial parts	Distilled water	23.35
	95% Ethanol	20.4
Rhizomes	Distilled water	28.5
	95% Ethanol	26

3.2 Preliminary Phytochemical Screening:

The results of the preliminary investigation for secondary metabolites are shown in Table 2. The (+) mark indicates a positive test result, while the (–) mark indicates a negative result. All extracts contain Flavonoids, Anthraquinones, Tannins, Coumarins, and Saponins.

Table 2: the results of preliminary investigation tests for secondary metabolites in *R. conglomeratus* extracts

Secondary metabolites	Test name	APA[1] extract	APE[2] extract	RA[3] extract	RE[4] extract
Flavonoids	Aluminum chloride	+	+	+	+
	Shinoda	+	+	+	+
Anthraquinones	Borntrager	+	+	+	+
	Modified Borntrager	+	+	+	+
Tannins	Ferric chloride	+	+	+	+
	gelatin	+	+	+	+
Coumarins	Fluorescence	+	+	+	+
Saponins	Foam	+	+	+	+
Alkaloids	Dragendorff	–	–	–	–
	Mayer	–	–	–	–
	Hager	–	–	–	–
	Wagner	–	–	–	–
Cardiac glycosides	Keller-Kiliani	–	–	–	–
	Kedde	–	–	–	–

[1]*aerial parts aqueous extract,* [2]*aerial parts ethanolic extract,* [3]*rhizomes aqueous extract,* [4]*rhizomes ethanolic extract.*

3.3 Total phenolic content

The total phenolic contents of R. conglomeratus extracts ranged between 279.86 ± 3.02 GAE/g DE and 502.55 ± 1.36 GAE/g DE (Table 3). The rhizomes' ethanolic extract had the highest phenolic content followed by RA, APE, APA extracts respectively.

3.4 Total flavonoids content

The aerial parts' ethanolic extract of *R. conglomeratus* had the highest flavonoids content (46.82 ± 0.394) mg QE/g DE, while the lowest flavonoids content was found in the rhizomes' aqueous extract (8.45 ± 0.504) mg QE/g DE (Table 3).

3.5 Total Anthraquinones content

The total anthraquinones contents of *R. conglomeratus* extracts ranged from 1.26 ± 0.093 mg RhE/g DE, to 6.71 ± 0.106 mg RhE/g DE. The rhizomes' ethanolic extract had the highest anthraquinones content, while the aerial parts' aqueous extract had the lowest content (Table 3)

Table 3: Total phenols (TPC), flavonoids (TFC), and Anthraquinones (TAnC) content in *R. conglomeratus* extracts

***R. conglomeratus* extract**	TPC mg GAE[1]/g DE[2]	TFC mg QE[3]/g DE	TAnC mg RhE[4]/g DE
APA	279.86 ± 3.02	28.54 ± 0.661	1.26 ± 0.093
APE	381.71 ± 2.85	46.82 ± 0.394	3.35 ± 0.062
RA	431.10 ± 1.57	8.44 ± 0.504	4.55 ± 0.125
RE	502.55 ± 1.36	12.30 ± 0.986	6.71 ± 0.106
[1]gallic acid equivalent, [2]dry extract , [3]quercetin equivalent, [4]rhein equivalent			

3.6 In vitro antioxidant activity assessment

3.6.1 DPPH scavenging activity:

The free radicals scavenging (RSA%) for each extract and gallic acid are shown in Figure 2. The IC50 values for gallic acid and *R. conglomeratus* extracts are also mentioned in Table 4. The most effective extract was the rhizomes' ethanolic extract, with an IC50 = 5.40 ± 0.380 mg/L. While aerial parts' aqueous extract had the lowest efficacy, with an IC50= 43.36 ± 0.474 mg/L.

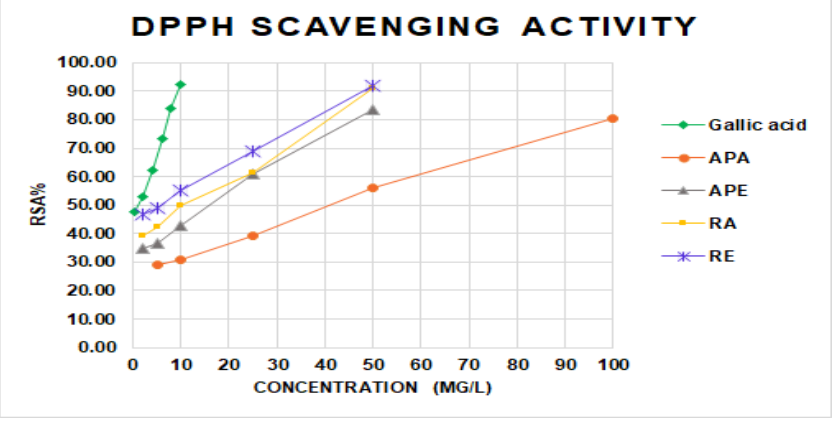

Figure 2: DPPH free radical scavenging activity (RSA%) of *R. conglomeratus* extracts and gallic acid

Table 4: RSA% and IC50 values of *R. conglomeratus* extracts and gallic acid standard in DPPH assay

***R. conglomeratus* extract**	**RSA% after 30 minutes at C = 10 mg/L**	**RSA% after 30 minutes at C = 50 mg/L**	**IC50 (mg/L)**
APA	30.90 ± 0.22	80.27 ± 0.32	43.36 ± 0.474
APE	42.89 ± 0.40	83.57 ± 0.54	16.80 ± 0.385
RA	49.81 ± 0.19	91.26 ± 0.19	11.97 ± 0.189
RE	55.08 ± 0.15	91.87 ± 0.26	5.40 ± 0.380
Gallic acid	92.44 ± 0.18	—	1.23 ± 0.008

3.6.2 FRAP Assay

The ferric reducing power of the extracts and ascorbic acid was concentration-dependent (Figure 3). At a concentration of 200 mg/L, the ethanolic extract of rhizomes exhibited the highest reducing power, with the highest absorbance (0.230 ± 0.004), although lower than that of ascorbic acid (0.923 ± 0.003). The aerial parts aqueous extract had the lowest reducing power (0.198 ± 0.003) (Table 5).

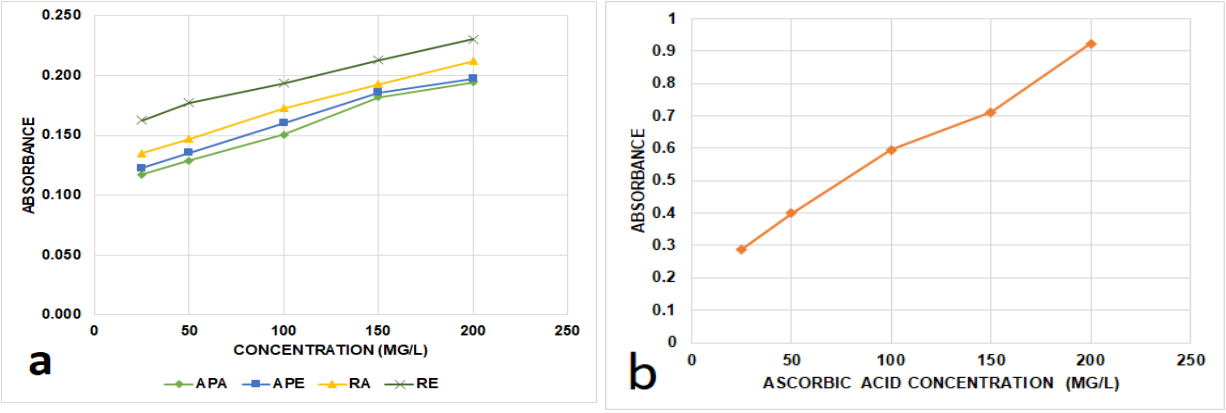

Figure 3: Ferric reducing power of (a) *R. conglomeratus* extracts, and (b) Ascorbic Acid standard

Table 5: Absorbance readings of R. conglomeratus extracts and Ascorbic Acid standard in FRAP Assay

Concentration (mg/L)	APA	APE	RA	RE	Ascorbic Acid
25	0.117 ± 0.003	0.123 ± 0.003	0.135 ± 0.003	0.163 ± 0.003	0.288 ± 0.001
50	0.129 ± 0.002	0.136 ± 0.003	0.147 ± 0.003	0.177 ± 0.004	0.400 ± 0.001
100	0.151 ± 0.001	0.160 ± 0.002	0.173 ± 0.002	0.194 ± 0.005	0.596 ± 0.002
150	0.182 ± 0.003	0.186 ± 0.001	0.193 ± 0.004	0.213 ± 0.004	0.711 ± 0.001
200	0.194 ± 0.002	0.198 ± 0.003	0.212 ± 0.002	0.230 ± 0.004	0.923 ± 0.003

3.6.3 Phosphomolybdate assay (Total Antioxidant Capacity):

The total antioxidant capacity of the *R. conglomeratus* extracts was expressed as the number of ascorbic acid equivalents (Table 6). The rhizomes ethanolic extracts had the highest antioxidant capacity (321.41 ± 6.94 mg AAE/g DE), while aerial parts aqueous extract had the lowest total antioxidant capacity (39.75 ± 3.16 mg AAE/g DE).

Table 6: Total antioxidant capacity of *R. conglomeratus* extracts in phosphomolybdate assay

R. conglomeratus extract	Total Antioxidant Capacity (mg AAE/g DE)
APA	39.75 ± 3.16
APE	174.33 ± 5.32
RA	264.56 ± 3.31
RE	321.41 ± 6.94

3.7 Statistical analysis:

Table 7 shows the correlation coefficients between TPC, TFC, TAnC and the antioxidant activities of the studied extracts.

Table 7: Correlation coefficients

	Correlation coefficient (r^2)		
	TPC	**TFC**	**TAnC**
IC50 (DPPH)	0.9263	0.1411	0.8671
FRAP	0.8541	0.5286	0.9108
TAC	0.9838	0.3355	0.955

DISCUSSION:

In this study, the extracts from aerial parts and rhizomes of *R. conglomeratus* Murr. were analyzed for the first time. The approach involved investigating the presence/absence of some secondary metabolites, determining the total content of phenols, flavonoids, and anthraquinones, and evaluating the antioxidant activity using three in vitro antioxidant assays: DPPH, FRAP, and TAC.

3.8 Yields of extraction

The four extracts prepared from *R. conglomeratus* aerial parts and rhizomes showed different extraction yields. The rhizomes extracts had higher yields than the aerial parts extracts, indicating that rhizomes contain a higher percentage of metabolites. The aqueous extracts of each plant part gave a higher extraction yield than ethanolic extracts, because the extraction solvent significantly affects the quality of the extracted compounds[31]. This suggests that the extracted substances are of a high polarity and predominantly present in the form of salts or glycosides which tend to dissolve more in water.

3.9 Phytochemical analysis

Preliminary phytochemical screening reactions revealed that all studied extracts of *R. conglomeratus* are rich in various secondary metabolites including, phenols, flavonoids, anthraquinones, tannins, coumarins, and saponins. The positive results of Shinoda's test indicated the presence of flavones and hydroxyflavones compounds[32], while the positive results of Borntrager's and modified Borntrager's tests indicated the presence of anthraquinones in both free aglycone and glycoside forms. While alkaloids and cardiac glycosides were absent in all extracts. Our results were similar to those reported by Mekonnen *et al.*[33] on ***R. abyssinicus*** rhizomes in Ethiopia, Gebrie *et al*.[34] on ***R. steudelii*** roots in Ethiopia, and Jaradat *et al.*[35] on ***R. rothschildianus*** leaves in Palestine. Whereas Ammar *et al*.[36] (Egypt) reported the absence of saponins and coumarins in the aerial parts of ***R. vesicarius*** and ***R. pictus***, and Hafaz *et al*. (Egypt) reported the presence of alkaloids in roots and shoots of ***R. dentatus***, ***R. pictus***, and ***R. vesicarius***, and the absence of coumarins in the roots of the three mentioned species[37].

The quantitative estimation of phenols, flavonoids, and anthraquinones revealed that the highest levels of total phenols and anthraquinones contents were observed in the rhizomes ethanolic extract (RE). Whereas, aerial parts ethanolic extracts (APE) had the highest content of total flavonoids. These results indicated that rhizomes contain higher amounts of phenols and anthraquinones than the aerial parts. While the aerial parts have a higher flavonoids content, which could be explained by the fact that flavonoids, especially hydroxyflavones, are mostly produced in flowers and vegetative organs to attract pollinators, or as a defense against environmental conditions[38]. We can also notice that the phenolic content was much higher than flavonoids and anthraquinones contents in all extracts. This could be due to the presence

of other phenolic metabolites such as tannins, stilbenes, lignans, and phenolic acids. From another perspective, the elevated phenols content could be related to the Folin-Ciocalteu method used, which depends on the reduction of the FC reagent by the phenolic compounds. However, the presence of reductants that contain hydroxyl groups other than phenols, such as reducing sugars, ascorbic acid, or proteins, will interfere and reduce the reagent as well. Therefore, this method might have overestimated the phenolic content in the studied extracts[39,40].

Compared with literature, previous studies on ***R. conglomeratus*** are very limited, Kilic *et al*. (Turkey) reported a lower TPC (43 mg/g dry extract) in the aqueous extract of leaves, but a higher TFC (119 mg/g)[16], and Marelli *et al.* (Italy) reported a lower TFC (15.5 mg/g) in leaves ethanolic extract[17]. Comparing with other *Rumex* species, a study on ***R. crispus*** (South Africa) confirmed the higher TPC in roots and ethanolic extracts, consistent with the findings of this study. The roots' ethanolic extract had a lower TPC (211.71 ± 9.65 mg GAE/g DE) but a higher TFC (45.19 ± 1.44 mg QE/g DE) compared to this study[41]. The roots' methanolic extract of ***R. roseus*** (Tunisia) also showed a lower TFC (10.81 mg QE/g DE) compared to the aerial parts (44.28 mg QE/g DE) and to the finding of this study[42]. TPC of the studied *R. conglomeratus* exceeded that of many other previously studied species, such as roots' ethanolic extract of ***R. crispus*** (Korea), (21.84 mg GAE/g/ DE)[43], aerial parts' methanolic extract of ***R. vesicarius*** (Algeria) (43.28 mg GAE/g/ DE)[44], and leaves and roots ethanolic extracts of ***R. dentatus*** (Egypt) (50.1 mg GAE/g/ DE) and (101.6 mg GAE/g/ DE) respectively[45].

Anthraquinones have potent biological activities[46], the laxative and digestion-enhancing effects are the most known for these compounds. Recent studies have evaluated the effectiveness of naturally occurring anthraquinones as antioxidant agents, and against coronavirus based on their antiviral and immune-boosting properties, in the context of finding new effective and safe drugs for the COVID-19 pandemic[47]. In this study, the total anthraquinones content in extracts of *R. conglomeratus* was determined. The rhizomes ethanolic extract had the highest anthraquinones content, which represents **0.67%** of the dry extract's weight, and about **0.18%** of the dry rhizome's weight. This is consistent with previous studies that anthraquinones are concentrated mainly in the underground parts of *Rumex* plants[48]. Eom *et al*.[43] reported the TAnC content of ***R. crispus*** roots ethanolic extract (22.97 ± 0.026 mg/g DE) higher than *R. conglomeratus* extracts, and Seitimova *et al*. reported a higher TAnC of ***R. tianschanicus*** roots ethanolic extract (1.77%)[49]. Litvinenko & Muzychkina reported the TAnC of the roots of 7 *Rumex* species, the highest content was for ***R. aquaticus*** (2.13%), and the lowest in ***R. thyrsiflorus*** (0.71%)[50].

Differences in qualitative and quantitative phytochemical analysis between different species could be attributed to several factors including genetic makeup, environmental conditions, and climate factors such as temperature, humidity, exposure to ultraviolet radiation, drought and soil type. These factors can affect the plant's requirements and the synthesis rate of secondary metabolites. The extraction method, solvent, plant part under study, calibration method, and standards also play crucial roles in the outcomes of these studies[51,52].

3.10 In vitro antioxidant activity

In vitro antioxidant activity assays revealed that all extracts exhibit free radical scavenging activity and reducing power, which are positively correlated with extract's concentration. The rhizomes' ethanolic extract showed the highest antioxidant activity in all applied assays, although it was lower than the standards. The antioxidant activities can be attributed mainly to the secondary metabolites, especially the predominance of polyphenols. The antioxidant activity increased with the ascendant contents of phenols and anthraquinones with a high correlation (refer to Table 7), suggesting that the effectiveness is highly related to them. The increase in flavonoids content in each plant part has been accompanied

by an increase in the antioxidant activity as well. Phenols, flavonoids, and anthraquinones are potent secondary metabolites that are recognized for their strong antioxidant activity through various mechanisms, they can directly scavenge free radicals or act as reducing agents, hydrogen or electron donors, and/or metal ions chelators[53]. The nature of bioactive compounds and their structures greatly affect the antioxidant activity[3], the increasing number of hydroxyl groups and the attachment of side chains to the aromatic rings give a higher antioxidant capacity of phenolic compounds[53]. Therefore, further research and isolation of chemical compounds responsible for the activity are highly recommended to confirm the results.

According to the findings of this study, *R. conglomeratus* extracts, especially the rhizomes, are rich in natural antioxidants, and could be used to prevent the body from serious health conditions related to oxidative stress. They can also be used in food industry as food preservatives, or as laxatives and digestion enhancers due to the anthraquinones content. The daily dose of anthraquinones as laxatives, ranges between 15 to 30 mg/day[54,55] . Therefore, it can be suggested to use *R. conglomeratus* rhizomes as laxatives at a dose ranging between (9 – 17) grams per day, or to use the dried ethanolic extract at a dose of (2 – 4.5) grams per day, one to three times a week at most. However, subsequent studies must be conducted to confirm the safety and effectiveness of the drug within this framework or other medicinal applications.

4. CONCLUSIONS

This study represents the first report on the chemical constituents and antioxidant activity of the aerial parts and rhizomes of *Rumex conglomeratus*. The findings revealed that *Rumex conglomeratus*, particularly the rhizome, is a reliable source of secondary metabolites and rich in phenols, flavonoids, and anthraquinones, with strong antioxidant activity as free radical scavengers and reducing agents. The rhizomes ethanolic extract had the highest antioxidant activity, which was highly correlated to the content of phenols and anthraquinones. Therefore, the results suggest using *R. conglomeratus* extracts as potent antioxidants that could protect the body from serious health conditions, or even as laxative agents or digestion-enhancers. However, further research is essential to confirm their safety and efficacy, emphasizing the importance of continued exploration into isolating and identifying biologically active compounds using a variety of chromatographic methods such as HPLC and LC-MS.

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Conflict of interest:

The authors declare no conflict of interest.

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نباتُ الحمّاض التّفِه المنتشر برّياً في سوريا: تحليلٌ كيميائيٌّ نباتيّ وتقييمُ الفعاليّة المضادَّة للأكسدة في الزُّجاج لخلاصاتِ الأجزاءِ الهوائيّة والجذامير

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ملخص

استُخدِمَ نبات الحمّاض التَّفِه *Rumex conglomeratus* Murr. (من الفصيلة البطباطية Polygonaceae) على نطاقٍ واسع شعبيّاً لعلاجِ العديد من الأمراض مثل الأمراض الجلديّة، الالتهابات، الإمساك، والسّرطان. وتعود الاستخدامات الشعبيّة والفعاليات الحيوية بشكلٍ رئيسيّ لغنى نباتاتِ جنس الحمّاض بالمستقلباتِ الثّانويّة الفعّالةِ حيوياً. تمثّل هذه الدِّراسة التقرير الأوّل للتّركيبِ الكيميائيّ والفعاليّة المضادّة للأكسدة للخلاصات المحضّرة من الأجزاء الهوائيّة والجذامير لنبات *Rumex conglomeratus*.. تمَّ في هذه الدّراسة تحضير الخلاصات المائيّة والإيثانوليّة، وأُجريت اختبارات الكشف الكيميائيّ الأوليّة، بالإضافة إلى تحديدِ إجمالي المحتوى من الفينولات، الفلافونوئيدات والأنتراكينونات، وتقييمِ الفعاليّة المضادّة للأكسدة باستخدام طرائق لونية يقاس فيها الامتصاص بجهاز UV-VIS spectrophotometer. أظهرت النّتائج أنّ *R. conglomeratus* مصدرٌ غنيٌّ بالمستقلباتِ الثّانويّة. وامتلكت خلاصةُ الجذامير الإيثانوليّة أعلى محتوى من الفينولات (502.55 ± 1.36 mg GAE / g DE) والأنتراكينونات (6.71 ± 0.106 mg RhE/g DE) كما امتلكت أعلى فعاليّة كاسحة لجذور DPPH الحرّة (IC50 = 5.40 ± 0.380 mg/L)، وقدرةً إرجاعيّة في اختبارِ FRAP (0.230 ± 0.004 at 200 mg/L) واختبار القدرة الكلّيّة المضادّة للأكسدة (321.41 ± 6.94 mg AAE / g DE). وكانت الفعالية المضادّة للأكسدة مرتبطة بشكل قوي بكلٍّ من المحتوى الفينولي والأنتراكينوني. تشير هذه النّتائج إلى إمكانيّة استخدام خلاصات نبات الحمّاض التَّفِه كمضادّ أكسدةٍ قويّ، أو حتى كعاملٍ ملّيّن. ومع ذلك من الضّروريّ إجراء المزيد من الأبحاث المستقبليّة لتحرّي المأمونيّة والفعاليّة، مع التّأكيدِ على مواصلةِ الاستكشاف لعزل وتحديد المركّبات الكيميائيّةِ الفعّالة حيوياً باستخدام طرائق الكروماتوغرافيا المختلفة، وتقييم العديد من الفعاليّات الحيويّة المُحتملة لخلاصات هذا النّبات الواعد.

الكلمات الدالة: الحماض التفه .Murr؛ الفصيلة البطباطية؛ الفينولات؛ الأنتراكينونات؛ الفلافونوئيدات؛ الفعالية المضادة للأكسدة.

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