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Phytochemical content and antioxidant activities of Rhubarb (*Rheum emodi*)

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ABSTRACT: Medicinal plants have varying natural products and several antioxidants. Antioxidants play a principal function to protect against destruction caused by oxidative stress (OS), *Rheum emodi* is not an exception, in which it is reported to have compounds that possess antioxidant activity, like polyphenolic compounds. In addition to that, other compounds have proven to have antidiabetic, antimicrobial, antifungal, cytotoxic, hepatoprotective, and nephroprotective activities. This study aimed to quantify the water extract of a powdered plant of *R. emodi* to evaluate its ability to scavage free radicals. Total phenolic, total flavonoid contents, and reducing ability were measured to consider possible sources of future novel antioxidants in plants. The TPC, TFC, and reducing power assay increased with increasing concentrations of *R. emodi*. At 1000 μg/ml, the absorbance ranged from 1.437 for TPC, 1.602 for TFC, and 1.638 for reducing power assay, which is more than the absorbance of the standards at the same concentration. The extracted content of reducing power, phenolic, and flavonoid compounds were higher than the standard pyrogallol, quercetin, and ascorbic acid.

1. INTRODUCTION

Changing the equilibrium between activated oxygen species (ROS) and antioxidants found in the body can cause oxidative stress that will cause chronic disease (Ighodaro & Akinloye, 2018; Khan et al., 2013). All systems (human, plants, and other biological systems) are powerful with an antioxidant defense mechanism that scavage free radicals in the body (Ighodaro & Akinloye, 2018), but sometimes this system is insufficient (Khan et al., 2013), and the immune system cannot reduce the free radicals alone, for that external antioxidant supplement is needed (Zargoosh et al., 2019). Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants, but due to their toxicity and DNA damage, their usage was rusticated (Zargar et al., 2011). Lately, floral resources with strong antioxidants and low toxicity have been used (Zargoosh et al., 2019)· (Zargar et al., 2011). *R. emodi* is one of the plants that has been used as an antioxidant because of its Scavenging Activities possessed from its phytochemical constituents (Chai et al., 2012).

R. emodi has also been known by the name rhubarb, this plant belongs to the Polygonaceae family, Rheum genus, and it is species called *R. emodi* (R. Singh et al., 2017) . *R. emodi* is widely distributed in China, Nepal, and India (Tayade et al., 2012). It is also endemic in the western and central Himalayan region (R. Singh et al., 2017). According to folk medicine, *R. emodi* is used to treat many diseases (Malik et al., 2016; Zargar et al., 2011) and according to the different constituents separated from *R. emodi*, we can predict the way it is used to treat a specific disease. For example, Anthraquinone (R. Singh et al., 2017)· (Hu et al., 2014; Kusmardiyani et al., 2016)and Phytosterol give *Rheum emodi* Anti-inflammatory activity and Antihyperglycemic activity respectively, Flavonoid and Phenolic glycosides that they work as Cardioprotective and Antihyperlipidemic respectively (Kusmardiyani et al., 2016). Along with these compounds, R. emodi has antioxidant and cytotoxic activities that could also be a reason for these therapeutic properties (Chai et al., 2012) and its antimicrobial activity (P.P. Singh et al., 2013). Other phytochemicals that have been identified (Chai et al., 2012) are anthrones c-glycoside (R. Singh et al., 2017) · (Hu et al., 2014), stilbenes, oxanthrone, ethers, ester, lignans, carbohydrate, and oxalic acid (R. Singh et al., 2017). In addition, naphthoquinones, rutin, rheinal, rhein 11-O-b-D-glucoside, torachrysone 8-O-b-D-glucoside, epicatechin, auronols (carpusin and maesopsin), the sulfated anthraquinone glycoside sulfemodin 8-O-b-D-glucoside, b-asarone, and some stilbene compounds (e.g., rhaponticin) have also been isolated (Hu et al., 2014).

In traditional medicine, it is mainly socked in water, and apportion is given to the patients; this study aims to determine

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the scientific basis of traditional uses of *R.emodi*. Examination of the antioxidant activity through reducing power assay, phenolics, and Flavonoids quantities of the water extract of the whole plant and compare it with reference compounds.

2. EXPERIMENTAL PART

Plant Collection and Identification: The plant was bought from Libyan markets and identified in the botany department at Benghazi University

Preparation of the leaf extract: 20 g of the fine-grained plant was added to 125 mL distilled water in a 250 ml flask then heated in a water bath at 70°C for 20 minutes. The plant biomass is separated through Whatman No.1 filter paper and concentrated with a rotary evaporator (RE2000). The extract was kept in a dry clean container until the analysis.

Total Phenolic content : The colourimetric method was used to determine the TPC according to the (Singleton et al., 1999) method, with some adjustments. The TPC contents in *R. emodi* water extract were calculated using the standard curve plot equation and expressed as mg pyrogallol equivalent /g of the dried plant material. The samples were measured in triplicates.

Reducing power assay : Oyaizu method (Oyaizu, 1986) was used to determine reducing power assay. The samples were measured in triplicates.

Total Flavonoid Content : Aluminum chloride colorimetric technic applied to determine TFC (Zhishen et al., 1999) (Chandra et al., 2014). The TFC contents in *R. emodi* water extract were calculated from the standard curve equation and expressed as mg quercetin equivalent /g of the dried plant material. The samples were measured in triplicates.

3. RESULT AND DISCUSSION

Total Phenolic content: TPC present in the plants possess redox properties that make them behave as antioxidants (Razali et al., 2019). TFC was estimated by (F.C) reagent, in which phenolates are oxidized by F.C which lead to the production of the blue complex of molybdenum-tungsten, this was detected spectrophotometrically at 765 nm (Gaikwad-Samdani et al., 2012). The absorbance value varied from (1.375± 0.021 at 100 μg/ml of pyrogallol) to (1.437± 0.006 at 500 μg/ml of pyrogallol). The quantity of TPC was calculated by a regression equation of a standard curve, y = 0.0009x + 0.1441, R^2 = 0.964, and ranged from 854.791 to 897.847 mg GPE per g of dry extract weight. The phenolic content in *R. emodi* water extract was higher than the n-butanol, ethyl acetate, n-hexane, dichloromethane, and water extracts reported by the study done by Park lee (Park & Lee, 2021). They reported that the lowest TPC is in the n-hexane extract of *R. emodi* was 27.76 mg GAE/g, while the highest amount is seen in ethyl acetate was 209.21 mg GAE/g. In addition, a study made by Singh and Chaturvedi (R. Singh & Chaturvedi, 2018) showed a lower TPC of 124.64 and 92.8 3μg GAE/mg for Fruits and rhizome extracts, respectively. Finally, the TFC of the methanolic and water extracts made by Ashok Kumar et al. (Kumar et al., 2011) showed lower values (36.44 μg GAE/mg and 14.30 μg GAE/mg for methanolic and water extract, respectively at a concentration of 200 μg/ml) than our results at the same concentration (Table 1).

Table 1
Total phenolic cotenant for pyrogallol and *Rheum emodi* water extract

Conc. "μg/ml"	Mean ± Standard Deviation		TPC as pyrogallol equivalent "mg/g"
	Pyrogallol	**Extract**	
200	0.292±0.005	1.375± 0.021	854.791
400	0.494±0.003	1.382± 0.012	859.652
600	0.797±0.007	1.406± 0.001	876.319
800	0.857±0.002	1.436± 0.001	897.152
1000	1.022±0.005	1.437± 0.006	897.847

Reducing Power assay: Reducers (i.e. antioxidants) present in a sample lead to the conversion of the ferricyanide complex used in this method to the /ferrous form ($Fe^{+3/}$ Fe^{+2}), and as a result of that, we measured Perl's Prussian blue formation at 700 nm. In which increased absorbance denotes the presence of higher reducing power (Barrosmaria & Queirós, 2007). The results compared to Ascorbic acid and revealed a higher reducing power assay for our sample. In which 200 ppm of our extract has almost the same Ferric reducing antioxidant assay of 1000 ppm of the ascorbic acid. This higher amount of reductone could react with reactive oxygen or nitrogen species to stabilize and terminate radical chain reactions indicating that the *R. emodi* water extract contains a high amount of flavonoids and polyphenols. Moreover, there are no available studies from other researchers to compare our data with it (Table 2).

Table 2
Reducing power assay for ascorbic acid and *Rheum emodi* water extract

Conc. "μg/ml"	Mean ± Standard Deviation	
	Ascorbic acid	**Extract**
200	0.293±0.012	0.929± 0.004
400	0.382±0.032	1.006± 0.004
600	0.445±0.008	1.462± 0.001
800	0.693±0.10	1.516± 0.004
1000	0.992±0.005	1.638± 0.009

Total Flavonoid content : Flavonoids are classes of natural products belonging to secondary metabolites with antioxidant activity due to OH groups in different numbers and positions (Aryal et al., 2019). The reaction with sodium nitrite determined TFC, the colored flavonoid-aluminium complex was produced using aluminium chloride. The colored complex was detected by spectrophotometer at 510 nm (Gaikwad-Samdani et al., 2012). The results were derived from the standard curve y= 2166x - 329.23, R^2 = 0.985. The flavonoid content in the extract ranged from 2.425 at a concentration of 200 μg/ml to 4.625 at a concentration of 1000 μg/ml, which is more than the reference at the same concentration. Research by Singh and Chaturvedi reported a TFC of (82.37 ±0.12 μg quercetin equivalent (QE) mg^{-1}) (R. Singh &

Chaturvedi, 2021), which is lower than what we got in our study. Also, an older study of Singh and Chaturvedi showed a TFC of 165 in leaves and 137.96 in rhizomes and callus (μg quercetin equivalent (QE) mg−1) using methanol as solvent, and these data were lower than what we get by the minimum concentration (200 μg/ml) (Table 3).

Table 3
Total flavonoid content of Quercetin and *Rheum emodi* water extract

Conc. "μg/ml"	Mean ± Standard Deviation		TFC as Querctin equivalent "mg/g"
	Quercetin	**Extract**	
200	0.236±0.003	0.661± 0.079	2.425
400	0.337±0.026	0.677± 0.084	3.056
600	0.442±0.087	1.127± 0.089	3.712
800	0.542±0.004	1.207± 0.039	4.338
1000	0.588±0.006	1.602± 0.046	4.625

4. CONCLUSION

The antioxidant activity of *R. emodi* water extract was confirmed by TPC, reducing power assay, and TFC. Compared to the references pyrogallol, ascorbic acid, and quercetin, the *R. emodi* had higher contents, which means they are better antioxidants. This variation in TPC, reducing power assay, and TFC could be due to geographical region, plant extraction time and conditions, extraction methods, and solvent evaporation methods. The increasing results may extend the study to the isolation of the bioactive compounds and measuring the antibacterial activity.

5. FUNDING

All the experiments proceeded in Benghazi University labs, and all the chemicals were provided by Benghazi University.

CONFLICTS OF INTEREST

Authors declares no conflict of interest.

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AUTHOR CONTRIBUTIONS

AS, NMEA - Research concept and design; AS, NMEA, YFL, SGET - Data analysis and interpretation; AS, NMEA, YFL, SGET - Writing the article.

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