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Article

Antioxidants and antibacterial activity of *Glycyrrhiza glabra* extract

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ABSTRACT

This study aimed to examine the antioxidants and antibacterial activity of *Glycyrrhiza* root, stem and leaf extract. The antioxidant activity was determined by measuring total phenolic content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH). The antibacterial activity was determined using the paper disc method against two bacteria, namely *Staphylococcus aureus* and *Escherichia coli.* Results showed part of the plant effect of the antioxidants and antibacterial activity. The results showed that the root sample had significantly ($P < 0.05$) higher total phenol content and antioxidant activity than leaves and stem samples. The TPC value of leaves extract 211.64mg GAE/g dry extract and DPPH 86.36 % was estimated. The antibacterial activity of MeOH extract against the *Staphylococcus aureus* zone of inhibition was 21.37 mm for root extracts and 8.30 mm against *E. coli.* The result showed that *Glycyrrhiza* root, stem and leaf extract were effective against both the bacteria tested with high concentrations. The *Glycyrrhiza* root, stem and leaf extract can be used to control infectious diseases and prevent oxidative damage.

Keywords: Glycyrrhiza glabra, DPPH, TPC, Antibacterial activity

INTRODUCTION

Natural product concerns to the primary and secondary metabolites. Since the origin of life, human beings depend on nature for their basic needs, such as shelter, food, clothing, means of transportation, and medicines [1]. Through trial and error, traditional healers found that some plants had healing power, and this knowledge was passed down through the generations. This knowledge is systematized and used in Ayurveda, Tibetan medicine, homeopathy, and Unani system [2]. Medicinal plants have minimal toxicity, are cost-effective, and are pharmacologically active; hence, they provide an easy remedy for many human ailments compared to synthetic drugs, which are subject to adulteration and side effects [3]. Antioxidants are compounds capable of delaying, retarding or preventing auto-oxidation. The importance of natural antioxidant constituents in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers and consumers as the future trend is moving toward functional food with specific health effects [4]. The beneficial health-related effects of certain phenolic compounds and their potential

antioxidant properties, especially when they are present in large quantities in foods, are important to consumers. Concern has been expressed about the potential toxicology of the long-term effects of commonly used synthetic antioxidants t-butyl hydroquinone, butylated hydroxyanisole, and butylated hydroxytoluene [5]. Therefore, several extraction procedures have been developed to replace commercially available synthetic antioxidants with natural sources. Extraction procedures were also developed to get more information about the plants that help to identify their quality. Different solvents have been used to extract antioxidants from plants, but no single procedure has proven superior to others. The commonly used solvents for extracting antioxidants from plant materials are acetone, ethanol, and methanol, separately or in aqueous mixtures [6]. The current study focused on the collection of traditionally used Glycyrrhiza root, stem and leaf extraction of plant secondary metabolites, and performed the antioxidant and antibacterial activity

MATERIALS AND METHODS

Sample collection and preparation of Glycyrrhiza extract

The root, stem and leaves of Glycyrrhiza glabra were obtained from farms north of Nasiriyah City, Iraq. The Glycyrrhiza samples were cleaned, cut into small pieces, and then oven-dried at 60 ^{0} C for 24 h. The dried sample was then pulverized using a mechanical grinder, passed through a 250 μm mesh, and then stored at 4^{0}C until use. In the extraction process, approximately 1 g of Glycyrrhiza samples were weighed in universal bottles and 10 ml of 50% acetone as solvent was added.

DPPH radical scavenging activity assay

The DPPH assay method of [6] was modified to determine antioxidant activity using Trolox as the standard. 3 ml methanol DPPH solution (40 mg/L) was mixed with 100 μl sample extract for assays. Samples were incubated in the dark at room temperature for 30 min, and then the absorbance of the solution at 517 nm was measured.

Determination of total phenolic content

The total phenolic content was estimated using the Folin-Ciocalteu method [6] using gallic acid as the standard. A 100-μL aliquot of plant extract was oxidized with diluted Folin-Ciocalteu reagent (500 μL). After 5 min, the mixture was neutralized with 1 ml sodium carbonate (7.5%, w/v) and incubated for 2 hours before reading absorbance at 765 nm.

Antibacterial assay

Staphylococcus aureus and Escherichia coli were used in the experiment. Mueller Hinton agar was used in the antibacterial assay. Glycyrrhiza root, stem and leaf extract were dissolved in methanol to obtain a concentration of 40μg/10μL. Antibacterial assays were conducted using the disc diffusion method previously described by [7]. Negative controls were prepared using the same solvent to dissolve the Glycyrrhiza root, stem and leaf extract. Zones of inhibition around the discs were measured in mm. The experiment was repeated in triplicate, and the mean diameter of the inhibition zones was calculated.

Statistical analysis

Data were expressed as the means of three independent experiments. Statistical comparisons of the results were performed by one-way ANOVA and Two-way ANOVA using SPSS ver.23. Significant differences ($P<0.05$) among the root, stem and leaves extract were analyzed by L.S.D.'s triplicates range test [8].

RESULTS

Based on DPPH estimates in Figure 2, the leaf sample had the highest percentage of antioxidant activity, the root sample had the highest, and the stem sample had the lowest. A significant difference ($P < 0.05$) between the leaf, root and stem TPC was approximately 211.64، 196.94 and 90.27 mg/100 g. The differences between the antioxidant content of Glycyrrhiza glabra root, stem, and leaf extract can be attributed to their differences in phenolic contents and compositions as other non-phenolic antioxidants in the samples. These differences in TPC results compared to other researchers may be related to the different types of cultivars and the different antioxidant extraction methods used. DPPH assays are often used to determine the capacity of primary antioxidants in samples, as these primary antioxidants react to scavenge free radicals from the DPPH solution. Hence, the formation of the free radical initiation chain is prevented, and the diffusion chain is destroyed by donating a hydrogen atom or electron. The bleaching of DPPH uptake by a test compound represents its ability to scavenge free radicals generated independently of any enzymatic system or transition metals. Compared to other methods, this method is widely used to assess antioxidant activities over a relatively short period. The antioxidants react with DPPH, a stable free radical, and convert it to 1, 1-diphenyl-2-(2, 4, 6-trinitrophenyl) hydrazine. The degree of discoloration indicates the scavenging potential of an antioxidant complex. As shown in Figure 2, the highest activity was in the leaf sample (86.36%), followed by moderate activities in the root sample (83.13%) and then the stem (58. 42%)

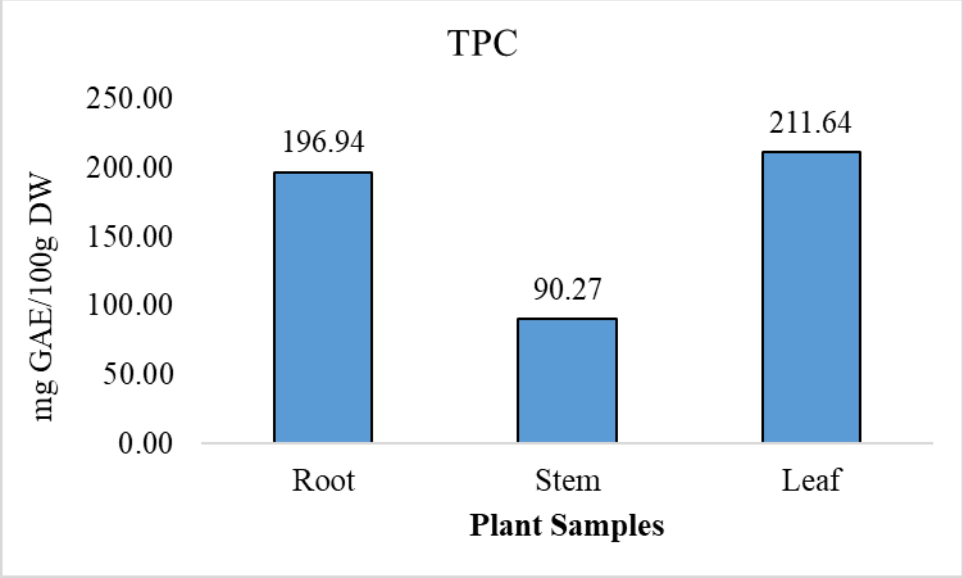

Figure 1: Mean (n=3) Total phenolic content of *Glycyrrhiza* root, stem and leaf extract

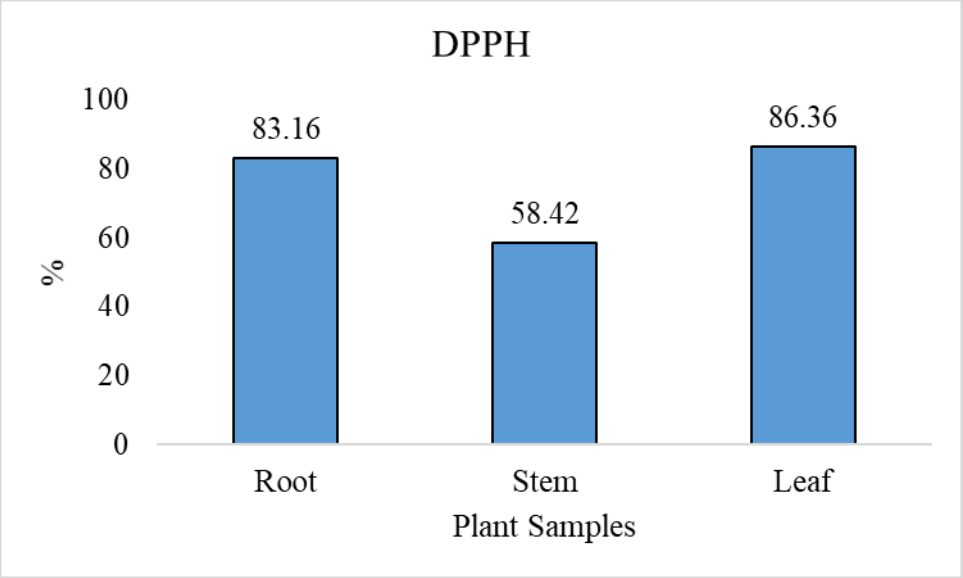

Figure 2: Mean (n=3) DPPH of *Glycyrrhiza* root, stem and leaf extract

Antibacterial activity

The antibacterial activity of crude methanol extract of *Glycyrrhiza* root stem and leaf extract was evaluated using the paper disc diffusion method against two types of bacteria: *Staphylococcus aureus* and *Escherichia coli*. Table 1 and 2 shows the results of antibacterial activities of three parts of *Glycyrrhiza* samples. This work found that antibacterial had significant differences ($p<0.05$) between both broccoli samples. Based on the result, the acetone extract of *Glycyrrhiza* root stem and leaf extract at a concentration of 100, 200 and 300 mg/ml showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Therefore, a higher concentration of antibacterial agent was essential to inhibit some of the Gram-negative bacteria.

Samples	**Concentrations mg\mL**			**Mean**
	100	**200**	**300**	
Root	18.07 ± 1.85	19.87 ± 1.33	21.37 ± 0.81	19.77
Stem	6.67 ± 1.06	7.07 ± 2.60	10.17 ± 3.34	7.97
Leaves	13.43 ± 1.30	16.17 ± 2.18	16.93 ± 0.76	15.51
Mean	12.72	14.37	16.16	

L.S.D = 3.22

Table 1: Effect of *Glycyrrhiza* root, stem and leaf extract on *Staph. Aureus*

Samples	Concentrations mg\mL			Mean
	100	200	300	
Root	3.86 ± 0.30	5.03 ± 0.45	8.30 ± 0.75	5.73
Stem	0.00 ± 0.00	3.93 ± 0.30	4.70 ± 0.75	2.88
Leaves	2.67 ± 0.61	4.56 ± 0.40	6.00 ± 1.00	5.73
Mean	2.18	4.51	6.33	

L.S.D = 0.98

Table 2. Effect of *Glycyrrhiza* root, stem and leaf extract on *E.coli*

DISCUSSION

Antioxidant compounds react with the Folin-Ciocalteu reagent, and the reaction can be performed to measure the concentration of phenolic groups [9]. TPC has been identified due to its strong association with antioxidant activity in different parts of Glycyrrhiza glabra root, stem, and leaf [10]. Moreover, factors such as root ripening, agricultural climate and post-harvest storage conditions influence the polyphenol content in fruit [11]. Further phenolic compounds are involved in adhesion binding, protein and cell wall binding, enzyme inactivation, and intercalation into the cell wall and/or DNA during the inactivation of pathogens [12]. The bacterial inhibition depends upon the extraction solvent used, part of the plant and organism tested. A previous study reported that although the fruit extracts of some seeds had antibacterial activity against *E. coli*, they had no effect against some other bacteria [13]. Another study reported that fruit and leaf extracts of some plants had an antibacterial effect against *S. aureus*, but their leaf extracts do not exhibit any influence against *E. coli* [14,15].

CONCLUSION

The results of the present investigation revealed that the plant extract of G. glabra contains pharmacologically active substances with antibacterial properties. Therefore, these Glycyrrhiza plant is rich in some antioxidants, and antibacterial activity is essential for human health. The Glycyrrhiza can be used to control infectious diseases and prevent oxidative damage.

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