



Detection of Total Flavenoids, Reductive Ability, and Anti-microbial in *Glycyrrhiza glabra* and *Achillea* Medicinal Plants.

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Abstract

According to WHO, 80% of the medicine in the global market are from plants. The Glycyrrhiza and Achillea are very promising plants. Three experiments were done for the plants. The total flavonoids, was higher in *G.glabra* than *Achillea*. In the second experiment, reductive ability was very effective in scavenger the free radical specially, in high doses. In the third experiment, the antimicrobial of the three types of bacteria isolated from two sources assess using extracts from plants.

Keywords: *Achillea* spp, *Glycyrrhiza* spp, medicinal plants, total flavonoids, reductive ability, antimicrobial activities

Introduction

The majority of people rely on their folk medicine (medicinal plants and other materials) for their everyday health care needs. It is also a fact that one quarter of all medical prescriptions are based on substances derived from plants or plant-derived synthetic analogs, and according to the World Health Organization (WHO), 80% of the world's population, especially those of developing countries, rely on plant-derived medicines for their healthcare [1]. This is depending on the fact that medicinal plants typically contain mixtures of different chemical compounds that may act individually, or in synergy to improve health. Accordingly, they have subjected to an intensive investigation to reveal their pharmaceutical potentials [2]. Scientific studies confirmed that the medicinal plants have potentials, and presented in vitro and in vivo. Evidences that medicinal plants or their secondary metabolites have shown different biological effects with a wide range of pharmacological properties; for instance, immune stimulator,

antibacterial, antiviral, anti-inflammatory, anti-oxidant, anti-mutagenic, anticancer, and many other properties [3-5]. Two plants had been used in this study: Licorice (*Glycyrrhiza glabra* L.; Family: Papilionaceae/Fabaceae) is a traditional medicinal herb grows in the various parts 2 of the world. It is a very sweet, moist, soothing herb that detoxifies and protects the liver and is also a powerful anti-inflammatory finds applications in arthritis and mouth ulcers. The plant is 2m height under shrub, The roots are long, cylindrical, thick and multibranch [6] very sweet. Te other plant used is The genus *Achillea* is represented by about 85 species throughout the world and 42 of them are found in the flora of Turkey; 23 of these are endemics [7, 8]. *Achillea* spp used for healing wounded soldiers during the Trojan War (1). Their herbal parts are used in folk medicine for the treatment of several diseases, disorders and ailments [9]. These species have some properties and used in cosmetics, fragrances and agriculture [10].

Some *Achillea* spp. are used as antiinflammatory, antibacterial, cytotoxic and haemostatic agents. *Achillea millefolium* is used as an anthelmintic, antiinflammatory, antispasmodic, antiviral, contraceptive, diuretic, diaphoretic, emenagogue, antipyretic, laxative, stimulant and for head and throat ache, hysteria, rheumatism and stomach ulcer.[11, 12]. Flowers of *A. ageratum* are used in gastrointestinal disorders and aerial parts of the same plant were reported to have cytotoxic activity. For all the above reasons, we choose these two plants to study their active compounds and the reductive activity.

Materials and Methods

Preparation of Plant Extract Fifty grams of the plant leaf powder were, extracted with 80% methanol (250 ml) at 65°C for 3 hours using the soxhlet apparatus. The extract solution was concentrated under reduced pressure in a rotary evaporator to yield dried crude extract which, was frozen at -20°C until used [13]. Three doses of the extract were tested (100, 200 or 300 mg /ml) in vitro. The selection of concentration was based on a previous investigation [14]. To prepare these doses, the dried methanolic extract was, dissolved in a few drops of DMSO (Dimethyl sulfoxide) and then diluted with distilled water to the required volume. **Source of Bacteria isolate and identification:** Two sources for bacteria isolated had been used 1. Wound infection patients in Alkindi hospital Baghdad Iraq for proteus and klebsiella spp. 2. Enterobacter spp. 3 was isolated from skimmed milk "Novelac" for babies in local market. Vetik kit used to identify these bacteria . **Determination of Total Flavonoids** Total flavonoids content was spectrophotometrically determined in the methanolic extract of the tested plants as rutin (flavonoids standard) equivalent by aluminium chloride colorimetric method as described by [15]. The methanolic extract (3.2 mg) was dissolved in 5 ml of

50% methanol. Then, addition of 1 ml of a 5% (w/v) sodium nitrite solution. After 6 min, 1 ml of a 10% (w/v) aluminium chloride solution was added and the mixture was allowed to settled for 5 minutes before 10 ml of a 10% (w/v) NaOH solution was added. The mixture was completed to 50 ml with distilled water and mixed well. Then, the absorbance was measured at 450 nm with a spectrometer after 15 min. A similar procedure was applied to six concentrations (2.5, 5, 10, 20, 40 and 80 µg) of rutin, and from which a standard curve was prepared. The total flavonoids content (as Rutin) was determined using a curve-fitting equation of the standard curve which is $Y = 0.0012x + 0.1109$ $R^2 = 0.9317$ [16]. **Assessment of Anti-oxidant Activity in vitro** Anti-oxidant activity of the tested plants, methanolic extract was in vitro assessed through the evaluations, of reductive ability radical scavenging activity. **Reductive Ability preparation Method** The method described by [13] was adopted to evaluate the reductive ability, in which 1 ml of each concentration of the plant extract (0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mg/ml) was mixed with 1ml of 0.2M phosphate buffer (pH 6.6) and 1.5 ml of 1% potassium ferricyanide. Then, incubated at 50°C for 20 minutes. Then, 1ml of 10% Trichloro acetic acid was added to the mixture to stop the reaction. The mixture, was centrifuged for 10 minutes at 3000 rpm, and 2.5 ml of the supernatant was mixed with 2 ml of distilled water and 0.5 ml of freshly prepared 1% Ferric chloride. After that, the absorbance was measured at 700nm. The same procedure was applied to the Trolox solutions (standards). All tests were done in triplicates.

Results and Discussion

Three experiments represented in this study: The total flavonoids was assessment for both plants. The results in figure 1 indicate great differences between both plants used.

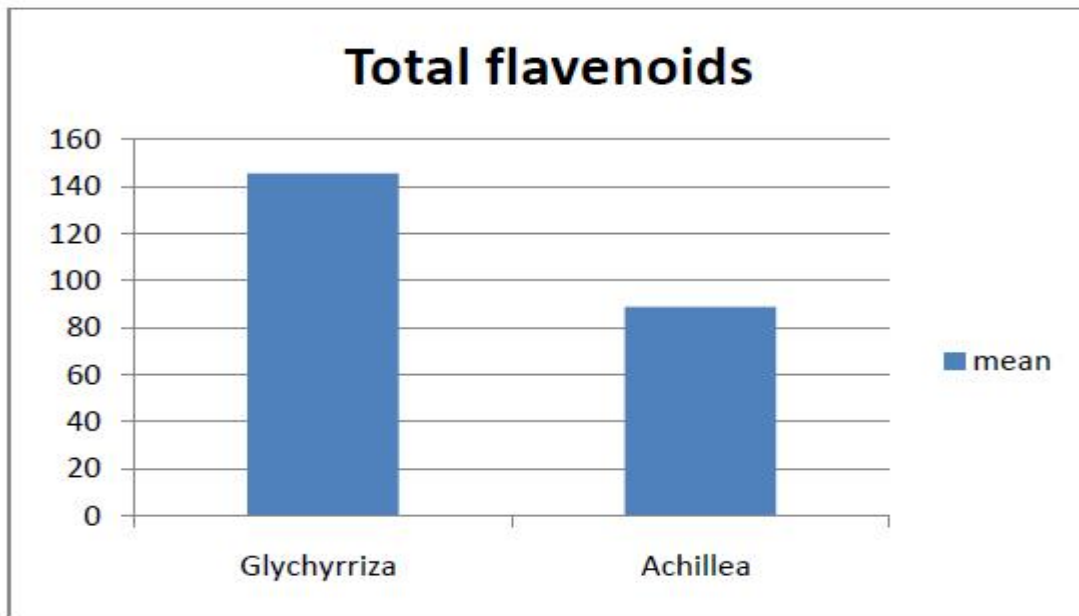


Figure 1: The total flavenoids for Glychyriza spp, Achillea spp.

In the second experiment calculation of reductive ability of the bacteria which had been used. As noted from figure 2 the R. ability decrease as the

concentration of the extract get low in both plants used.

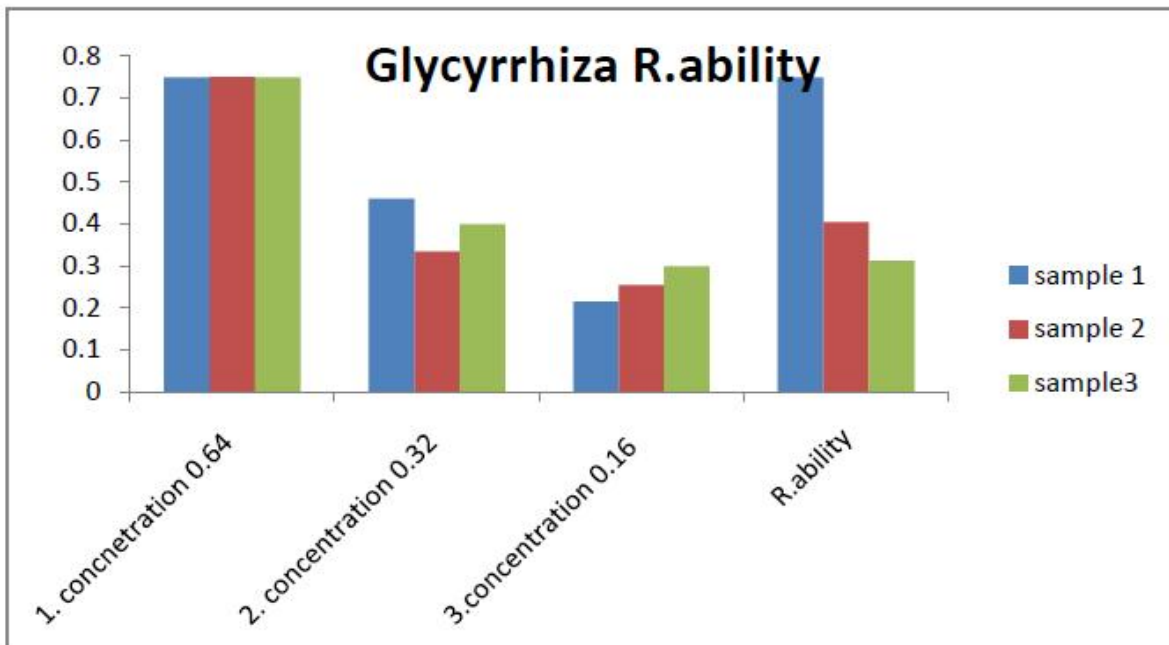


Figure 2 : Reductive ability for three different concentrations of Glycyrrhiza reductive ability

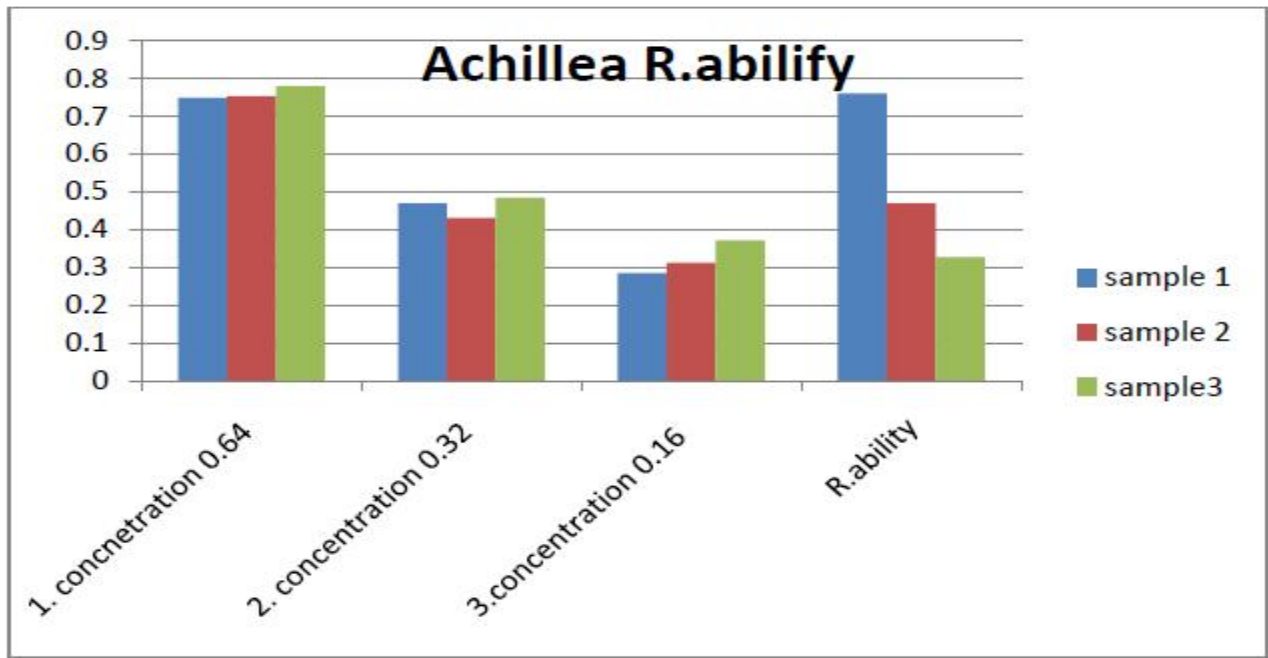


Figure 3 : Reductive ability for three different concentrations of Achillea reductive ability

The anti-microbial experiment, were the methanol extract of the two plants in concentration 300,400,500mg/l were tested against three types of

bacteria which are: Enterobacter sakasaki,E. corona,Klebsiella pheumonia, and proteus bulgaris. The results in figure 4 indicate:

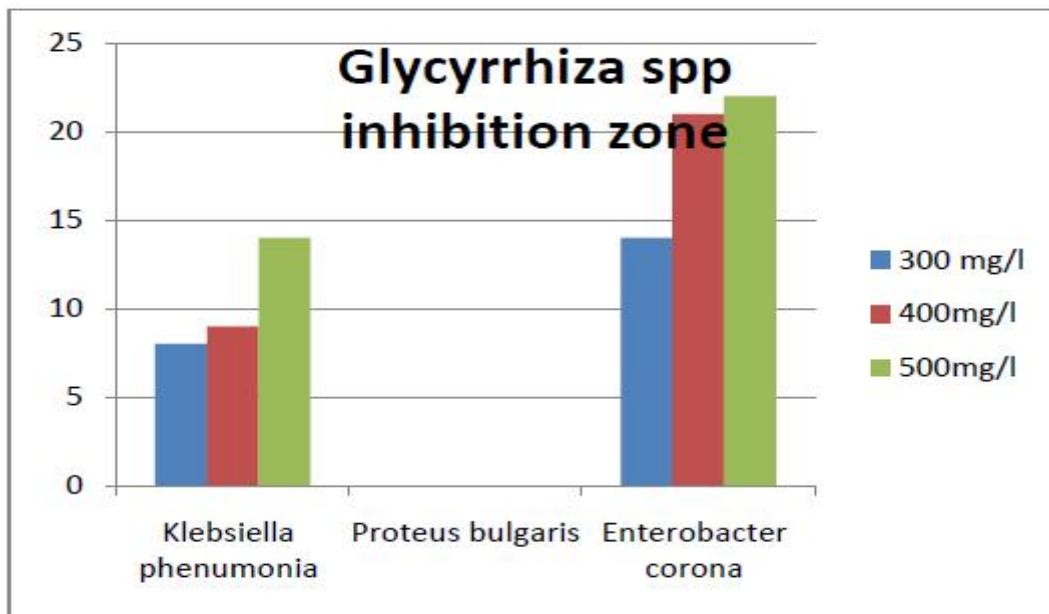


Figure 4: Inhibition zone of the three bacteria in Glycyrrhiza spp extract.

Large inhibition zone found in E.corona followed by K.phenumonia, while no inhibition indicate in P. bulgaris. However, the inhibition zone is increase as

concentration of the extract increased. Similar results found in Achillea spp. In figure 5, where all bacteria have inhibition zone even the proteus bulgaris.

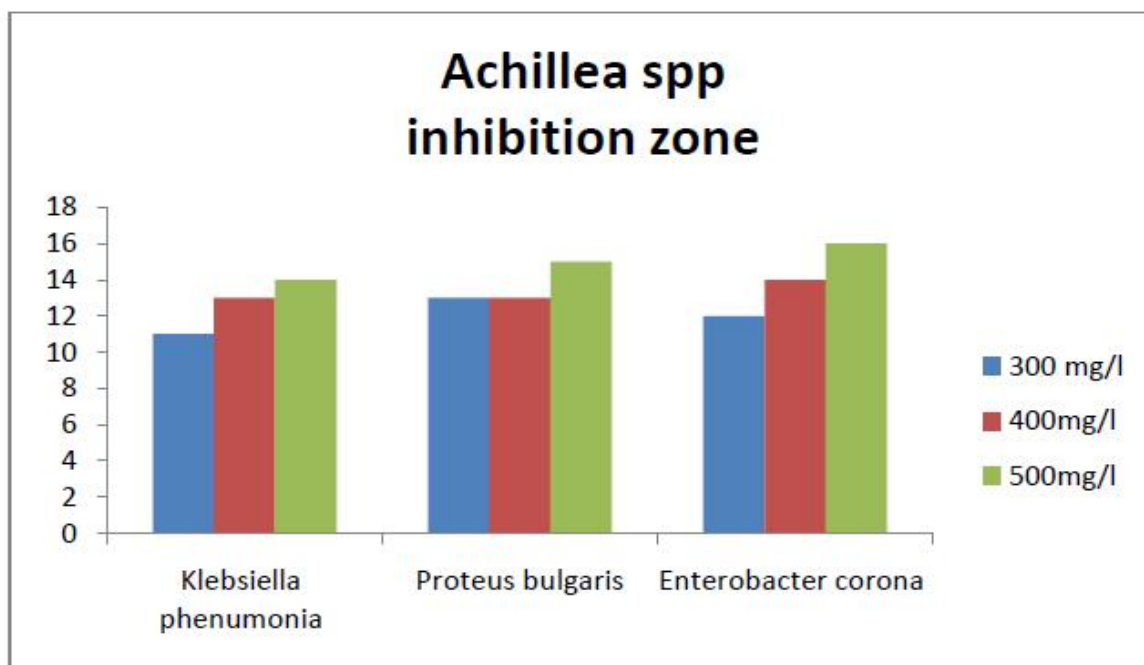


Figure 5: Inhibition zone of the three bacteria in Achillea spp extract.

A very narrow difference was found in A.spp, between the concentration affect in each bacteria as in figure 5 above.

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