

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

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Received: May 28, 2017    Accepted: June 26, 2017    Published: July 19, 2017

doi:10.5296/jbls.v8i2.11297    URL: <https://doi.org/10.5296/jbls.v8i2.11297>

## Abstract

According to WHO, 80% of the medicine in the global market are from plants. The *Glycyrrhiza glabra* and *Achillea mellefolium* are very promising plants. Three experiments were done for these plants. The total flavonoids, was higher in *G.glabra* than *A.mellefolium*. 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 mellefolium*, *Glycyrrhiza glabra*, medicinal plants, total flavonoids, reductive ability, antimicrobial activities

## 1. Introduction

The majority of people rely on their folk medicine for their everyday health care needs. It is a fact that one quarter of medical prescriptions based on substances derived from plants or plant-derived synthetic analogs. 80% of the world populations, especially those from developing countries, rely on their medicines on plants According to the World Health

Organization (WHO). (Gurib-Fakim, A., 2006). This is relying on the fact that medicinal plants contain mixtures of different chemical compounds that may act individually, or in synergy to improve health. Nevertheless, they have subjected to an intensive investigation to reveal their pharmaceutical potentials (Yang, Y. & et al., 2013).

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, anti-viral, anti-oxidant, anti-inflammatory, anti-mutagenic, anticancer, and many other properties (Chan, J. M. & et al., 2005; Butler, M. S. & Newman, D. J., 2008; Soladoye, M. O. & et al., 2010).

Two plants had been used in this study: Licorice (*Glycyrrhiza glabra* L.; Family: Papilionaceae/Fabaceae) is a traditional medicinal herb grown in the various parts of the world. It is an herb, which is sweet, moist, soothing that detoxifies and protects the liver and it is a powerful anti-inflammatory finds applications in arthritis and mouth ulcers. The plant 2m height, the roots are long, cylindrical, thick and multi-branched, very sweet (Scientific, C.o., I. R., 1985).

The other plant used in this experiment is The genus *Achillea millefolium* which is represented by about 85 species throughout the world and 42 of them are found in the flora of Turkey; 23<sup>th</sup> of these are endemics (Davis, P., & et al., 1988; Güner, A. & et al.). *Achillea* spp used for healing during the previous War (1). Their vegetative parts are used in folk medicine for the treatment of several diseases, disorders and ailments (Skočibušić, M. & et al., 2004). This plant has some properties and used in cosmetics, fragrances and agriculture (Senatore, F. & et al., 2005).

Some *Achillea* spp. was used as antibacterial, anti-inflammatory, cytotoxic and haemostatic agents. *Achillea millefolium* is used as a laxative, antiviral, anti-inflammatory, anthelmintic, antispasmodic, contraceptive, diuretic, diaphoretic, emenagogue, antipyretic, stimulant and for throat and head ache, hysteria, rheumatism and stomach ulcer. (Duke, J. A., 1992; Kırk çüoğlu, M. & et al., 2003). Flowers of *A. ageratum* are used in intestinal 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.

## 2. Material and Methods

### 2.1 Plant Extract Preparation

Fifty grams of the air-dried plant leaf powder were, extracted with 80% methanol (250 ml) at 65 °C for 3 hours using the Soxhlet apparatus according to (Sokmen, A., Jones & et al., 1999). The extract solution was concentrated under reduced pressure in a rotary evaporator to yield dried crude extract which, which was frozen at -20 °C until used (Fu, W. & et al., 2010).

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 (Kumar, A. & et al., 2010). 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.

### 2.2 Source of Bacteria Isolate and Identification

Two sources for bacteria isolated had been used 1. Wound infection patients in Al-kind hospital Baghdad Iraq for *proteus* and *klebsiella* spp. 2. *Enterobacter* spp. was isolated from skimmed milk "Novelac" for babies in local market. Vetik kit used to identify these bacteria .

### 2.3 Total Flavonoids Determination

Total flavonoids content was spectrophotometrically determined in the methanolic extract of the tested plants as Rutin (Total flavonoids) equivalent by aluminium chloride colorimetric method as described by (Sakanaka, S. & et al., 2005). 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 settle 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 Rutin content ( as total flavonoids) was determined using a curve-fitting equation of the standard curve which is  $Y = 0.0012x + 0.1109$   $R^2 = 0.9317$  (R. M. I. A. -E., 2015).

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

## 3. Method

The method described by (Fu, W. & et al., 2010) 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 1 ml of 0.2M phosphate buffer at pH 6.6 and 1.5 ml of 1% potassium ferricyanide. Then, the extract incubated at 50 °C for 20 minutes. 1 ml of 10% Trichloro acetic acid, was added to the mixture to stop the reaction. The mixture centrifuged for 10 minutes at 3000 rpm, and 2.5 ml of the supernatant mixed with 2 ml of distilled water and 0.5 ml of freshly prepared 1% Ferric chloride. After that, the absorbance measured at 700nm. The same procedure applied to Trolox solutions (standards). All tests were done in triplicates.

## 4. Results and Discussion

Three experiments represented in this study:

The total flavonoids was assessing for both plants. The results in figure 1 indicate great differences between both plants used. The flavonoids have an interest as dietary constituents; however in clinical studies indicate their roles in preventing cardiovascular disease and many kinds of cancer (Chu, Y. H. & et al., 2000). In other studies flavonoids and phenolics compounds have an efficient free radical scavenger activity besides some lipid peroxides

inhibitors (Egert, S. & Rimbach, G., 2011). Therefore, increasing flavenoids in both plants may be attributing to the increasing of reductive abilities of these plants (Franco, M. N. & et al., 2014).

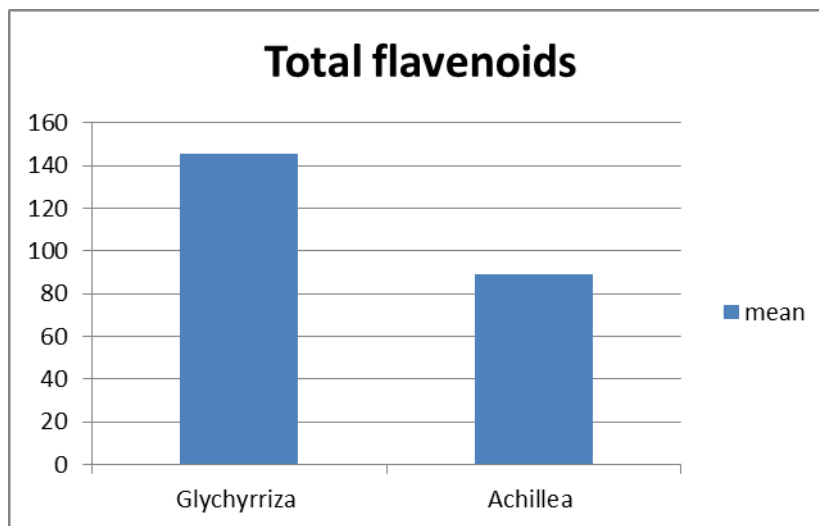


Figure 1. The total flavonoids for Glychrriza spp, Achillea spp.

In the second experiment calculation of reductive ability of the plants 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. It is, well known that free radicals can cause diseases by lipid, protein peroxidation or by DNA damage, however, many plants extracts have antioxidant activities which prevent free radicals from causes any disease. (Hsu, C.-Y. & et al., 2007). Moreover, phenols found a large quantities in plants and have antioxidant activity (Cocetta, G. & et al., 2015).

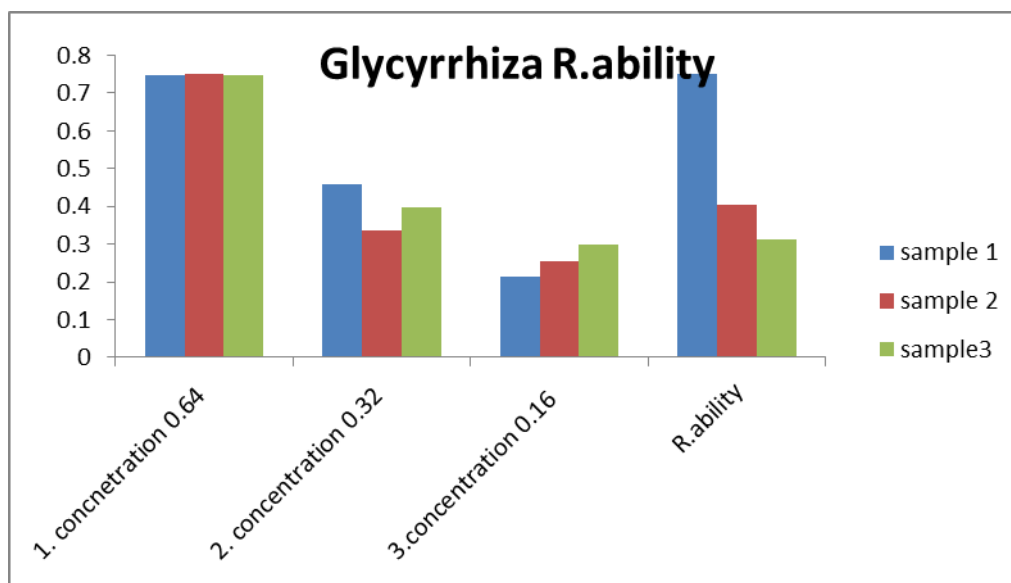


Figure 2. Reductive ability for three different concentrations of Glycyrrihza reductive ability

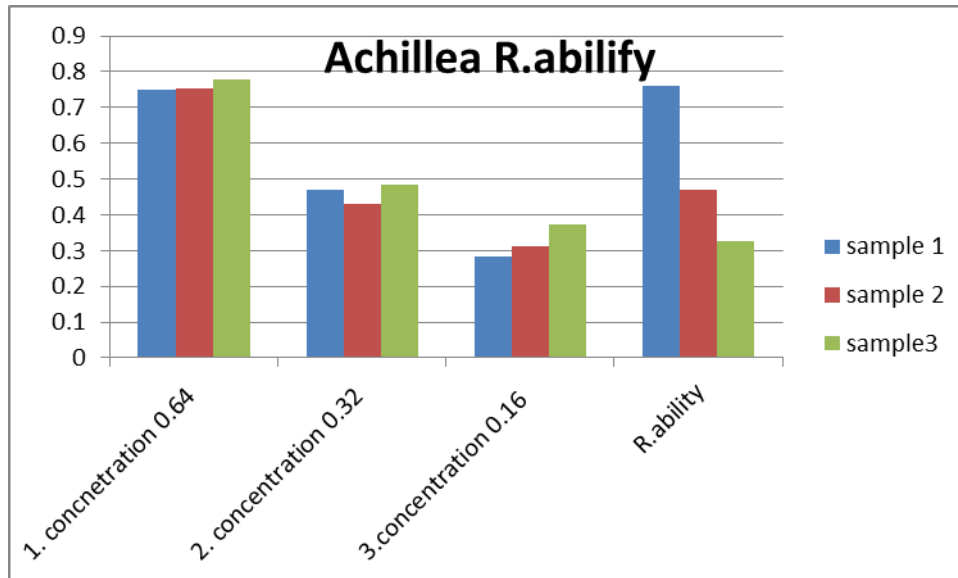


Figure 3. Reductive ability for three different concentrations of Achillea reductive ability

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:

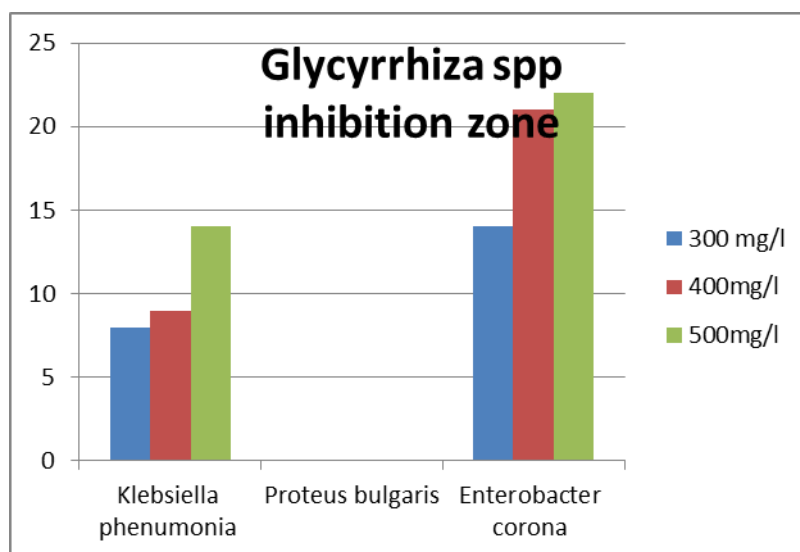


Figure 4. Inhibition zone of the three bacteria in Glycyrrhiza spp extract.

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*.

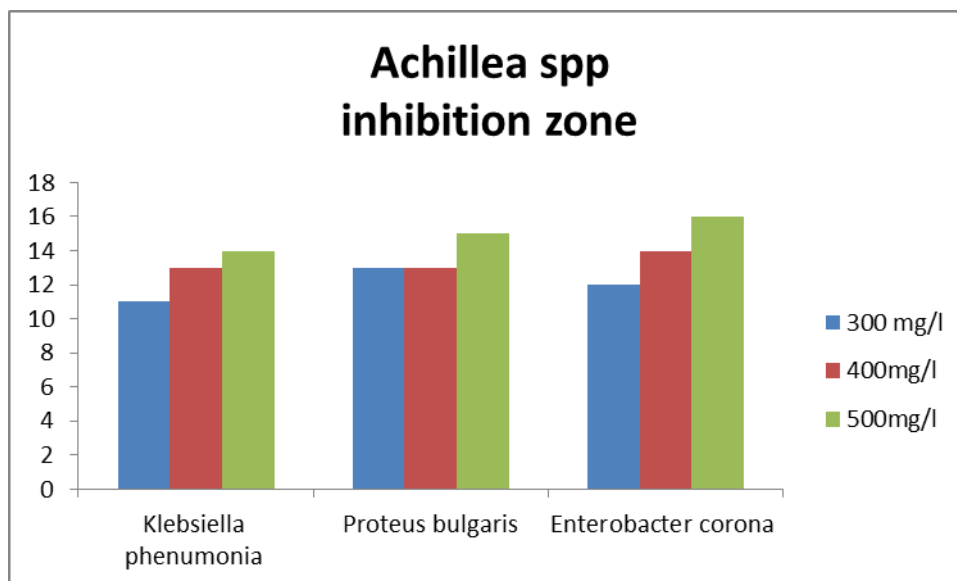


Figure 5. Inhibition zone of the three bacteria in Achillea spp extract.

A very narrow difference was found in A.spp, between the concentration affect in each bacteria as in figure 5 above. The two extracts in the above experiment, indicate high flavenoids which have good antimicrobial activities (Lakshmi T.). This activites may be linked to the presence of essential oil compounds which effect many bacteria in varying degree (Issabeagloo, E. & Abri, B., 2012).

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