



Antioxidant Potential of *Glycyrrhiza glabra* L. roots: *In-Vitro* Evidences

Varinder Singh¹ and Amit Kumar²

^{1,2}Chitkara College of Pharmacy, Chitkara University, Punjab-140401, India

¹varinder.9096@chitkara.edu.in

²amit.k@chitkara.edu.in (Corresponding Author)

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ABSTRACT

Background: The study was aimed to determine the mechanism of antioxidant effects of *Glycyrrhiza glabra* L. (GG) roots using in-vitro assays.

Methods: The various extracts of GG roots were prepared and evaluated for DPPH scavenging, reducing effects and nitric oxide inhibiting activities. Prepared extracts were screened for the presence of various phytochemicals and quantified on the basis of phytochemical present therein.

Results: The results showed that all the prepared extracts contained phenolic compounds. Also, extract showed appreciable antioxidant effects in all three assays employed. However, among prepared extracts, ethylacetate extract was found to have strong free radical inhibition, ferric reducing potential and nitric oxide inhibitory effects. The reason for high antioxidant activity in ethylacetate extract could be attributed to the significant amount phenol compounds present in it.

Conclusion: Evidently, GG's capacity to scavenge free radicals, reducing potential and inhibit nitric oxide contributes to its antioxidant effects and thus, could be a strong candidate for developing antioxidant based drug therapy.

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1. Introduction

Reactive oxygen species are produced in body during various metabolic processes such as mitochondrial respiration (Uttara, Singh, Zamboni & Mahajan, 2009). Under, altered pathological conditions, ROS production substantiate the endogenous antioxidant capacity and resulted in development of various disorders (Liguori et al., 2018). Therefore, oxidative stress is considered as a crucial player in the pathogenesis of wide array of diseases. Hence, reduction of oxidative stress seems to be a viable approach for the managing ailments.

Herbal drugs have been a great source of drugs as drugs of natural origin are being employed clinically (Veeresham, 2012). Literature has shown that various phytochemicals such as phenol, alkaloids, steroids, terpenoids, flavonoids etc exhibit strong antioxidant potential and thus are capable of managing cancer, diabetes, hypertension, neurodegeneration etc (Szymanska, Pospisil & Kruk, 2016; Unuofin & Lebelo, 2020). Therefore, exploring natural products for compounds exhibiting antioxidant properties has attracted attention of researchers. *Glycyrrhiza glabra* (GG) is one such natural product plant which has been reported to have neuroprotective, antidiabetic, anti-inflammatory, antiulcer, expectorant, antimicrobial and hepatoprotective effects (Pastorino et al., 2018). However,

the antioxidant effect of GG in different assays has not been systematically reported. Therefore, the present study is designed to evaluate GG for antioxidant activity using various *in-vitro* assays.

2. Material and Methods

The GG roots were procured from local market of Patiala, Punjab, India. The obtained roots were dried and powdered. The powdered material was extracted by maceration as described in Figure 1. Qualitative tests to identify different compounds in extracts was done as described by Singh et al., (2016). Quantitative estimation of phenol was performed as described by Singh et al., (2016).

2.1. Free Radical Scavenging Potential

The radical inhibitory effect of prepared extracts was determined by DPPH assay. The extracts were dissolved in methanol and its 10 – 70 µg/ml concentrations were prepared. One ml of each dilution was mixed with 1 ml solution of DPPH. The mixture was kept under incubation for 15 minutes and the absorbance was noted by UV spectrophotometer. Ascorbic acid (1-20 µg/ml) was used as standard drug. The percentage inhibition was calculated as described by Singh et al., (2016).

2.2. Reducing Potential

The reducing potential of prepared extracts was determined in terms of ferric reducing capacity. The extracts were dissolved in methanol and its 10 – 70 µg/ml concentrations were prepared. Each concentration was allowed to react with potassium ferricyanide in presence of trichloroacetic acid as described by Singh et al., (2016). Ascorbic acid was used as standard drug (Ahmed, Khan & Saeed, 2015).

2.3. Nitric Oxide Inhibitory Potential

The nitric oxide inhibitory effect of prepared extracts (10-70 µg/ml) was determined by Griess reagent as described by Basu & Hazra, (2006). Ascorbic acid was used as positive control.

3. Results and Discussion

Free radicals are very reactive molecules that are formed during interaction of oxygen or nitrogen with certain biomolecules. Based on the capacity of free radicals to accept or donate electron, they are called as oxidant and reductant, respectively. These reactive radicals (oxygen or nitrogen) have capacity to interact with various cellular molecules and shows deleterious effects such as DNA damage, apoptosis, mitochondrial dysfunction etc and thus, play significant role in the progression of various age and non-age related

disorders (Liguori et al., 2018; Pizzino et al., 2017). Nitric oxide is released by neurons, macrophages and endothelial cells and causes inflammatory pathways (Lubos, Handy & Loscalzo, 2008). Therefore, to prevent and manage health disorders, it is necessary to develop drugs that can counteract the action these energetic radicals.

In the present study, GG roots were tested whether it has free radical scavenging properties using in-vitro assays. *In-vitro* DPPH, ferric reducing antioxidant potential (FRAP) and nitric oxide inhibitory assays are widely used methods to screen drugs for antioxidant effects. These assays are easy to perform, accurate and give reproducible results. Furthermore, these methods depict the different antioxidant mechanisms. Therefore, these methods are selected in the present study to evaluate radical scavenging, reducing potential and nitric oxide inhibitory effects of GG extracts.

The gradient extraction of GG roots showed varied percentage yield of extracts. Since, methanol extract showed highest percentage yield, it is likely that GG roots contained high content of polar compounds (Table 1). The percentage yield and physical properties of prepared extract are given in Table 1. The phytochemical screening showed the presence of phenols in all the prepared extracts while carbohydrates were found to be present in aqueous extract only. Quantitative analysis of phenols in extracts showed that ethylacetate extract had highest amount (Table 2, Figure 2).

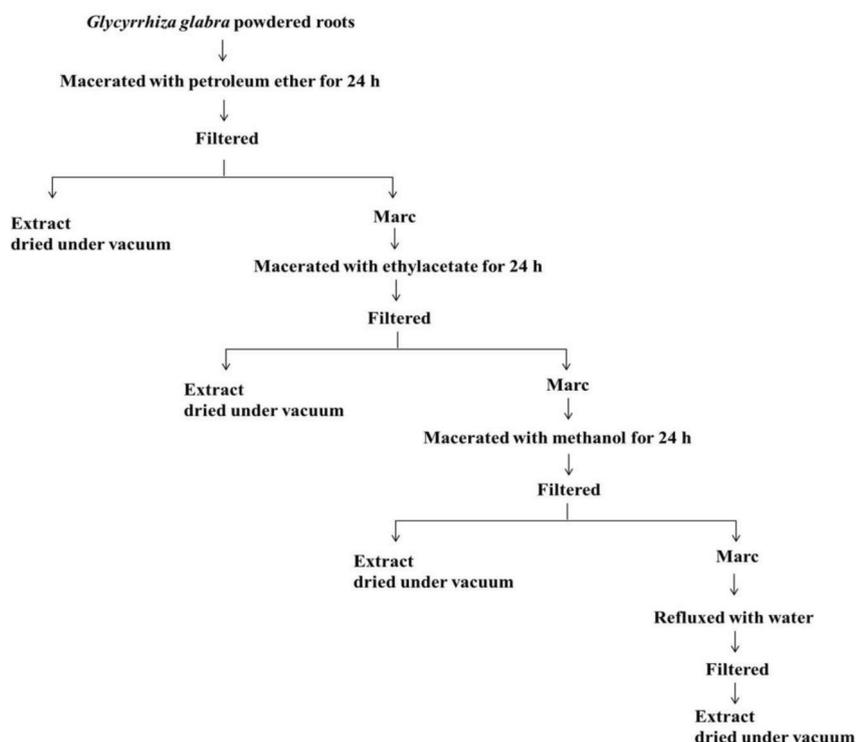


Figure 1: Extraction scheme.

Table 1: Percentage yield and physical properties of prepared extracts.

Extract	Colour	Consistency	Percentage yield (% w/w, air dried material)
Petroleum ether	Yellowish green	Semi-solid	1.02
Ethylacetate	Brown	Semi-solid	2.76
Methanol	Brown	Semi-solid	4.28
Aqueous	Yellowish brown	Semi-solid	3.11

Table 2: Phytochemical screening and total phenol content of prepared extracts.

Extract	Phytochemical screening	Total phenol content (mg/g of dried extract)
Petroleum ether	Steroids, terpenoids	Not determined
Ethylacetate	Terpenoids, phenols	2.4
Methanol	Phenols, flavonoids, carbohydrates	1.8
Aqueous	Phenols, flavonoids, carbohydrates	1.4

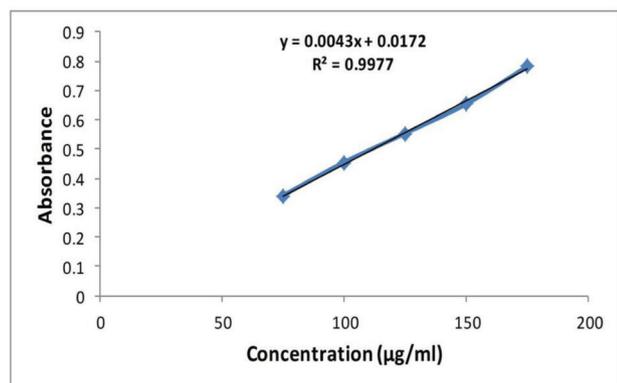


Figure 2: Standard plot for total phenol content.

In *in-vitro* antioxidant analysis, all the extracts exhibited antioxidant effects. However, ethylacetate extract showed marked antioxidant potential in all the three methods employed (Figure 3-5).

Phenols are well established antioxidant compounds and are reported to be beneficial in various diseases including, cancer, diabetes, neurodegenerative, kidney, liver, hypertension, cardiovascular disorders (Pandey & Rizvi, 2009; Dzialo et al., 2016). Keeping in view the beneficial effects of phenols, it is possible that higher phenol content in ethylacetate extract contribute to its antioxidant effects. However, these results warrant further studies including isolation of antioxidant compound and *in-vivo* investigations.

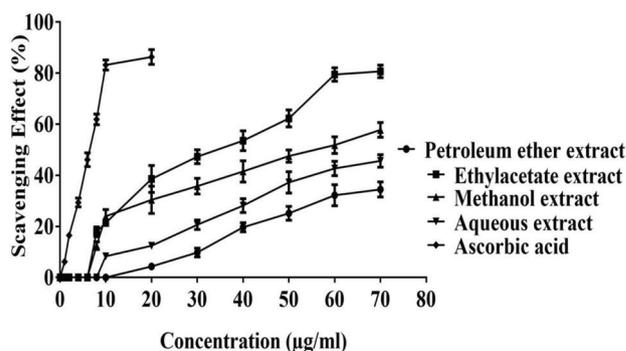


Figure 3: DPPH scavenging effects of extracts.

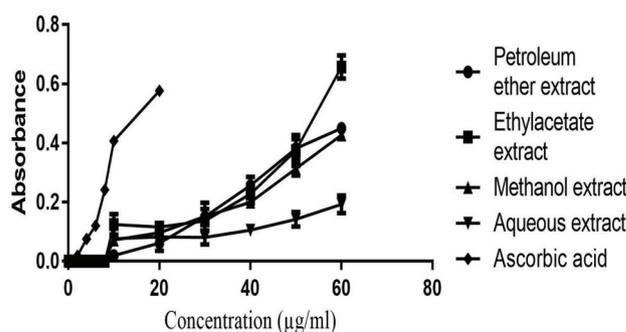


Figure 4: Ferric reducing potential of extracts.

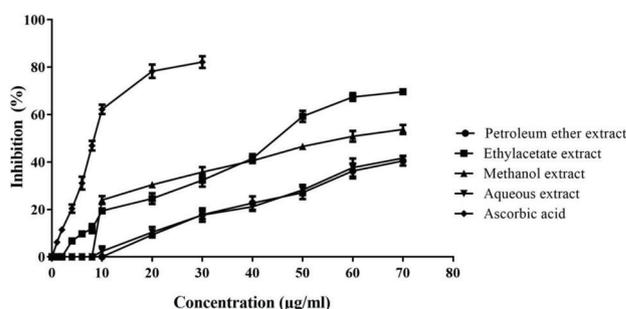


Figure 5: Nitric oxide inhibitory effects of extracts

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Conflict of Interest

There is no conflict of interest.

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Chitkara University, Saraswati Kendra, SCO 160-161, Sector 9-C,
Chandigarh, 160009, India

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