chemistry proceedings

MDPI

Proceeding Paper

Phytochemical Screening and Inflammatory Activity Evaluation of Hydroalcoholic Extract of *Glycyrrhiza glabra* Root †

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† Presented at The 28th International Electronic Conference on Synthetic Organic Chemistry (ECSOC 2024), 15-30 November 2024; Available online: https://sciforum.net/event/ecsoc-28.

Abstract: *Glycyrrhiza glabra* (licorice) belongs to the family Fabaceae and has a long story in traditional medicines and folk remedies to treat inflammation, arthritis, gastrointestinal problems and dyspepsia, Consequently, there is ongoing research into novel plants and herbal compounds possessing anti-inflammatory properties, aiming to uncover more potent alternatives while mitigating the potential toxicities associated with conventional anti-inflammatory medications. The aim of the present study was to evaluate the anti-inflammatory activity of Hydroalcoholic extract of *Glycyrrhiza glabra* root, in order to find new and more effective agents for the treatment of degenerative and inflammatory diseases. The activity of anti-inflammatory was assessed by the protein denaturation method using the standard drug Diclofenac. The phytochemical constituents identified were flavonoids, alkaloids, phenols, saponins, steroids, and terpenoids, with flavonoids, alkaloids, and phenols being the most abundant. The results showed that the Hydroalcoholic extract of *G. glabra* root have a very good inhibitory effect, with percentages of 81%, at a concentration of 10 g/L compared to Diclofenac (61.3%). Hydroalcoholic extract of roots exhibit attractive property anti-inflammatory, which can be attributed to the presence of secondary metabolites of different classes of compounds and can therefore, be considered a promising candidate for future application as alterntive therapeutic agents, particularly in the development of anti-inflammatory drugs.

Keywords: *Glycyrrhiza glabra*; Inflammation; Hydroalcoholic extract; Phytoconstituents

Citation: Zatla, A.T.; Hammoudi, A. Phytochemical Screening and Inflammatory Activity Evaluation of Hydroalcoholic Extract of *Glycyrrhiza glabra* Root. *Chem. Proc.* **2024**, *6*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: 15 November 2024

1. Introduction

In a context where chronic inflammatory diseases are on the rise, the search for effective and sustainable treatments has become essential. Natural anti-inflammatories derived from plant sources are gaining popularity due to their beneficial effects and often superior safety profile compared to synthetic drugs. Unlike the latter, which can lead to unwanted side effects and long-term complications, natural compounds offer a holistic approach to the treatment of inflammation [1,2]. Incorporating phytochemicals into health regimens allows inflammatory mechanisms to be targeted while minimizing the risks associated with conventional treatments.

The root of *Glycyrrhiza glabra*, commonly known as licorice, belongs to the Fabaceae family and has been used as a medicine and flavoring agent for over 400 years. This plant continues to play an important role in the pharmaceutical and food industries. Licorice extracts are widely used in functional foods and dietary supplements [3–5]. They also serve as flavoring agents in various products such as baked goods, ice cream, chewing gum, candy, and soft drinks [6].

Licorice has been used in traditional medicine for centuries and is valued for its sweet taste and health benefits [7,8]. Its roots and rhizomes have been used by ancient civilizations, including the Indians, Egyptians, Chinese, Greeks, and Romans, to treat various

ailments, particularly those related to the respiratory and digestive systems [9]. It is also beneficial in treating conditions such as epilepsy, fever, sexual weakness, rheumatism, paralysis, psoriasis, and jaundice [9]. In addition, it is effective against ailments such as gout, inflammation, acidity, bleeding, hiccups, and digestive and ophthalmic disorders, including gastralgia, headache, and pharyngodynia [9].

Phytochemicals detected in the hydroalcoholic extract of *Glycyrrhiza glabra*, such as flavonoids and saponins, have shown significant anti-inflammatory properties, thus offering a promising alternative to synthetic treatments [10].

This study aims to explore in depth the phytochemical profile of the hydroalcoholic extract of the root of *Glycyrrhiza glabra* while evaluating its anti-inflammatory activity, thereby contributing to the valorization of this plant in the health field and expanding the therapeutic options available for the treatment of inflammatory diseases.

2. Material and Methods

2.1. Collection of Plant Material

The roots of *Glycyrrhiza glabra* L. were collected in their natural habitat in March 2023. The identification and classification of *G. glabra* was confirmed by Dr. KAZI TANI Choukry, botanist affiliated to the Department of Pharmacy at the University of Tlemcen. The plant was stored in a dry place, protected from humidity and light. The roots were then cleaned, cut into small pieces and dried.

2.2. Preparation of the Hydroalcoholic Extract of the Roots of Glycyrrhiza glabra L.

Licorice extract was prepared by macerating 15 g crushed root in 100 mL hydroethanolic solution (70/30, *V*/*V*) for one week at room temperature. The extract was then filtered through Whatman No. 1 filter paper to remove plant residues and obtain a clear liquid. The filtered extract was dried in an oven at 45 °C for 3 days, to remove excess solvent. The extract obtained was then stored in opaque glass pillboxes, hermetically sealed, protected from light and humidity, and at low temperature.

2.3. Phytochemical Screening

The qualitative phytochemical screening was conducted using the following methods [11]:

2.3.1. Flavonoids

To detect the presence of flavonoids, a few drops of concentrated hydrochloric acid (HCl) and a few milligrams of magnesium (Mg) are added to 1 mL of hydroalcoholic extract. The presence of flavonoids is confirmed by the appearance of a red or orange color.

2.3.2. Alkaloids

The presence of alkaloids was detected using Meyer's reagent, with the appearance of a white precipitate as a positive result.

2.3.3. Saponosides

To 1 mL of hydroalcoholic extract, a few drops of sodium bicarbonate solution were added. The test tube was shaken vigorously and left to stand for 5 min. The formation of foam indicated the presence of saponins.

2.3.4. Tannins

To detect the presence of tannins, 1 mL of a 1% aqueous $FeCl_3$ solution was added to 1 mL of hydroalcoholic extract. The presence of tannins is revealed by the appearance of a blue-black color.

2.3.5. Polyphenols

To perform this test, 1 mL of a 2% aqueous $FeCl_3$ solution is added to 1 mL of hydroalcoholic extract. The appearance of a blue-black color indicates the presence of polyphenols.

2.3.6. Heterosides

1 mL of hydroalcoholic extract is mixed with 2 mL chloroform and 3 mL concentrated sulfuric acid. The presence of heterosides is revealed by the appearance of a reddish-brown color in the interface layer.

2.4. In Vitro Anti-Inflammatory Activity

The in vitro anti-inflammatory activity of the hydroalcoholic extract of the roots of *G. glabra* was studied using the egg albumin protein denaturation assay according to Fetni et al. (2020) with some modifications [12]. For each concentration of hydroalcoholic extract (2 mL) or standard (diclofenac), 2.8 mL of phosphate-buffered saline (PBS), pH = 6.5 was added, mixed with 0.2 mL of egg albumin (fresh). Samples were incubated in a 37 °C oven for 15 min, then immersed in a 70 °C water bath for 5 min. After cooling the tubes, the absorbance (level of protein precipitation) was measured at 660 nm in a spectrophotometer [13] and the percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ d'inhibition} = 100 \times [Abs_{sample}/Abs_{control} - 1]$$

The result is the average of three replicates.

This method evaluates the potential of the extract to inhibit the denaturation of proteins, hence indicating its anti-inflammatory properties.

3. Results and Discussions

The hydroalcoholic extract of the roots of *Glycyrrhiza glabra* L. produced a brown-colored powder with a yield of 16% relative to the initial dry mass of the plant. This result highlights the efficiency of the extraction process, which successfully concentrated the active compounds of the root into a solid form.

3.1. Phytochemical Analysis of the Hydroalcoholic Extract G. glabra

The results of the phytochemical tests performed to identify the different families of secondary metabolites are presented in Table 1. Phytochemical analyses of the hydroalcoholic extract of the roots of *Glycyrrhiza glabra* L. revealed a remarkable diversity of secondary metabolites, indicating the presence of different bioactive compounds. A significant presence of flavonoids, heterosides, saponins, alkaloids and polyphenols were observed, along with a moderate presence of tannins.

Table 1. Phytochemical test results.

Flavonoids	Alkaloids	Tannins	Saponosides	Polyphenols	Heterosides
+++	+++	++	+++	+++	+++

+++ Strongly positive, ++ moderately positive.

These results are in agreement with the study by Meghashri and Shubha Gopal, where extraction was performed from dried roots of *Glycyrrhiza glabra* using 80% ethanol followed by fractionation in multiple solvent phases [14]. The similarity in results highlights the efficiency of ethanol in the extraction of secondary metabolites from licorice. Several important factors influence phytochemical assays. Geography plays a crucial role, with environmental conditions specific to each region affecting the composition of plant extracts. For example, studies conducted in two different cities in Algeria, revealed

variations in the presence of flavonoids and alkaloids due to these environmental differences. In addition, the extraction method chosen, whether hydroalcoholic maceration, aqueous extraction or Soxhlet extraction, directly influences the metabolites detected. Finally, the physical form of the plant used (pieces versus powder) and the type of solvent (pure ethanol, hydroethanol, water) also play a crucial role in the chemical composition of the extracts.

3.2. In Vitro Anti-Inflammatory Activity

The in vitro anti-inflammatory activity of the hydroalcoholic extract of *G. glabra* roots and sodium diclofenac was analyzed using the protein denaturation method, as shown in Table 2. The results presented show a concentration-dependent inhibition of protein (albumin) denaturation by the samples. Sodium diclofenac was used as a reference drug at the same concentration. The results indicate that the hydroalcoholic extract of *G. glabra* roots has a very good inhibitory effect, reaching a percentage of 81% at a concentration of 10 g/L, compared to diclofenac, which showed an inhibition percentage of 61.3%.

Table 2. Percentages of inhibition of protein denaturation of the hydroalcoholic extract of *G. glabra* roots and sodium diclofenac at different concentrations.

	% Inhibition	
Concentrations (g/L)	**Sodium diclofenac (%)**	**Hydroalcoholic Extract of *G. glabra* (%)**
1	9.1 ± 0.2	12.6 ± 0.3
2,5	16.7 ± 0.6	20.3 ± 0.5
5	38.5 ± 1.1	60.8 ± 0.9
10	61.3 ± 0.3	81.1 ± 0.2

Samples and positive control were performed in triplicate (n = 3). SD = standard deviation.

This observation is consistent with recent research highlighting the efficacy of plant extracts, particularly those containing flavonoids and saponins, in modulating inflammatory responses. For example, studies have shown that flavonoids found in *Glycyrrhiza glabra* exert anti-inflammatory effects by inhibiting the release of pro-inflammatory cytokines and modulating cellular pathways involved in inflammation [15]. In addition, saponins are recognized for their ability to interfere with the activation of inflammatory mediators, reinforcing the idea that plant extracts may provide a viable alternative to conventional pharmaceutical treatments [16].

Further research highlights the potential of natural products in the treatment of chronic inflammatory diseases, which is particularly relevant given the increasing prevalence of these conditions. The side effects associated with NSAIDs, including gastrointestinal disturbances and cardiovascular risks, underscore the need to seek safer and more effective alternatives. Licorice extract, with its favorable safety profile and demonstrated efficacy, may represent a promising avenue for the development of anti-inflammatory treatments.

Comparative studies suggest that the bioactive compounds in *G. glabra* may also have synergistic effects when combined with other phytotherapeutic agents, broadening the available treatment options and offering new perspectives for the management of inflammatory diseases [17]. The discrepancies between the results of our study and those of previous studies may be due to the differences in preparation and extraction methods, as well as environmental conditions in different regions, which can significantly affect the concentration of active chemical compounds in licorice [18].

In summary, these findings underscore the potential of licorice root as a natural therapeutic agent for the treatment of inflammation, particularly in the context of the increasing demand for alternatives to synthetic treatments.

4. Conclusions

This study represents the first exploration of the phytochemical composition and anti-inflammatory activity of the hydroalcoholic extract of *Glycyrrhiza glabra* L. roots. The extract revealed the presence of various phytochemical compounds, including flavonoids, heterosides, saponins, alkaloids, polyphenols and tannins. It is particularly interesting to note that root extract showed greater anti-inflammatory power than diclofenac sodium, a commonly used synthetic anti-inflammatory drug. This is probably due to the diversity of secondary metabolites it contains, making it a promising candidate for future development as an alternative therapeutic agent, particularly in the creation of new anti-inflammatory drugs.

Author Contributions: Conceptualization, A.T.Z. and A.H.; resources, A.T.Z.; writing original draft preparation, A.T.Z.; writing review and editing, A.T.Z. and A.H.; visualization, A.H.; supervision, A.T.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available in this manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

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