Egypt. J. Chem. **Vol. 65,** No. 10 pp. 667 - 672 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Chemical Analysis and Antibacterial Activity of *Glycyrrhiza glabra* roots

Heyam G. Al Mousawi[1], Rasha Hadi Saleh[1], Aseel Mohammad Omran[1], Amal Talib Al Sa'ady[*1], Abdolmajid Ghasemian[2]

[1]College of Pharmacy/University of Babylon, Iraq

[2] Noncommunicable Diseases Research Center, School of Medicine, Fasa University of Medical Sciences, Fasa, Iran

Abstract

Glycyrrhiza glabra roots contain high nutritive value and substantial medicinal properties such as antibacterial, antioxidant, antimalarial, antispasmodic, anti-inflammatory and anti-hyper glycemic activities. Plant samples were collected from Babylon governorate, Iraq. Confirmed classification of *G. glabra* and preparation of root extract were implemented in the laboratories of college pharmacy/university of Babylon. Various extracts and chemical compounds were obtained and the antimicrobial effects of *G. glabra* was assessed against *Staphylococcus aureus*, *Escherishia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* using agar well diffusion method and concentration range of 500-3000mg/mL. Results of the chemical analysis of *G. glabra* root extracts proved that the aqueous extract contained saponin, flavonoids, glycosides, but lacked alkaloids and phenols. The ethanolic extract contained tannins and terpenoids. All tested isolates were sensitive to both extracts at concentration of 3000 mg/ml. *S. aureus* and *E. coli* showed higher sensitivity, maximum effective inhibition for *G. glabra* aqueous extract was found against *S.aureus*, whereby the minimum inhibition was recorded against *P. aeruginosa*. Regarding *G. glabra* alcoholic extracts, the highest effect was noticed against *P. aeruginosa*, while the lowest effect was noticed on *C.albicans*.

Keywords: *Glycyrrhiza glabra*, roots, chemical analysis, antimicrobial activity.

. Introduction

Because of the increasing antibiotic resistance, the demands have increased for plant-based drugs, herbal health products and herbal pharmaceuticals. Hence, medicinal plants became great important source of medical products [1,2,3].

Since long ago, *Glycyrrhiza glabra* has documented as one of the most common traditional medicines used in Europe and China. *G. glabra* is a herbaceous perennial belonged for family *Leguminosae* with traditional names "liquorice" or "licorice". there are various uses of liquorice roots in foods and medicines atributed for its numerous biological active compounds such as flavonoids, glycyrrhetinic acid, glycyrrhizin, chalcones, triterpenoid , tannins, pectins, asparagines, cortisol metabolism inhibitors, volatile oils, glycosides and isoflavonoids [4,5,6]. In the medical field, the powder and extract of liquorice roots commonly are used for treatment of cold, cough, asthma, sore throat and other respiratory disorders. Glycyrrhizinic acids have been used to cure atopic dermatitis, pruritis, and cysts due to the skin parasitic infestations. Glycyrrhizin can reduce the IgE-stimulating cytokines leading for interferance with the production of IgE [7,8,9,10].

Scientific evidences in the published studies have demonstrated high antibacterial activities of liquorice root extracts against bacteria and fungi. On the other hand, numerous biological components have been isolated from *G. glabra* roots with potential antimicrobial activities *in vitro*, such as glabridin, 40-methylglabridin, glabrol, 3-hydroxyglabrol, hispaglabridin A and B, gabrin, and glabrene. Glycyrrhizinic acids have been used to treat the eczema, itchy skin, and Cysticercosis [11,12,13]. Antiviral activity by Glycyrrhizin has been reported via inhibition of the viral attachment to the host cell, disruption with viral multiplication and can be used for prophylaxis treatment, such as treatment of chronic hepatitis C virus and HIV. Recently, reviewed evidence has indicated that another components of liquorice extracts have antiviral activities such as pyraziofurin, ribavirin, 6-azauridine and glycyrrhizin. The related studies emphasized, mycophenolic acid

*Corresponding author e-mail: amal.atiyah@yahoo.com.; (Amal Talib Al Sa'ady).

Receive Date: 20 January 2022, Revise Date: 01 March 2022, Accept Date: 31 May 2022

DOI: 10.21608/EJCHEM.2022.117422.5294

can be used against severe acute respiratory syndrome (SARS) virus [14,15,16].

2. Materials and Methods

2-1. Plant Samples collection

Roots of *G. glabra* were collected from Al-Hussain village in Babylon governorate, Iraq on March, 2021. Confirmed identification and classification of *G. glabra* and preparation of root extract were done in the pharmacognosy laboratory /college of pharmacy/university of Babylon. The roots were cleaned and cut into small pieces and dried.

2-2.Preparation of *Glycyrrhiza glabra* root extracts

The collected roots of licorice plant were dried and grinded with mechanical grinder. In glass flask 50g of the dried roots powder was dissolved in 500 ml of 70% ethanol for 24hrs. In another glass flask, 50g of the dried roots powder was dissolved in 500 mL of boiling water for 24hrs. The both flasks were put on the shaker for 6hrs. The extracts were sterilized by Millipore filter units[17]. The filtrated extract was dried at 57-60°C in oven and the dried extract powder was used to prepare alcoholic crude extract and crude aqueous extract.

2-3.Chemical analysis

2-3-1.Detection of Tannins

After preparing the aqueous solution of 1% of ferric chloride,we added an equal amount of plant extract, the appearance of abluish-green precipitate indicated the presence of tannins [18].

2-3-2. Detection of flavonoids

5ml of ethyl alcohol 95% was added to 1mL of plant extract in atest tube and placed in water bath for 30 minutes. Next a few drops of sodium hydroxide was added and the appearance of dark color indicated the presence of flavonoids [9].

2-3-3.Detection of saponins

5 ml of the extracted plant root was placed in a test tube, and a few drops of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 5 minutes. Foam formation indicated the presence of saponins [18].

2-3-4.Detection of Glycoside: by using Fehlan،k reagent which consisted of two solutions [9].

Solution (a)/ 7 g of hydrated copper sulfate dissolved in 100ml of distilled water. **Solution (b**)/ 35 g of potassium sodium tertarate and 10g of sodium hydroxide dissolve with D.w. Equal volumes of Fehlan،k reagent and plant exract were mixture in abioling for 15 minutes ,we noticethe appearance of a red sediment at the bottom test tube indicating the presence of glycosids.

2-3-5.Detection of terpenoids

1 ml of chloride antimon in 20% chloroform, was added to 5ml of plant extract. The appearance of white precipitate was an indication of the terpenes presence [9].

2-3-6.Detection of phenols

A mixture of 1% aqueos iron chloride and 1% of potassium iron cyanide was prepared and and an equal amount of plant extract was added. The appearance of greenish-blue color indicated the presence of phenols [9].

2-3-7.Detection of Alkaloids:

Alkaloids were detected by Meye،r's reagent which gives white precipitate as positive result [18]

2-4. Antimicrobial activity assay

glabra extract was investigated separately on Brain-Heart infusion Agar (BHIA) medium using well-agar diffusion method. The study was performed using 18-20hrs bacterial culture of *S. aureus*, *E. coli*, *P. aeruginosa*, and the fungi *C. albicans* standard strains.

2-4-1.Agar well diffusion method

The aqueous and alcoholic extracts of *G. glabra* were tested at various concentrations (500, 1000, 2000 and 3000mg/ml). A nutrient broth was inoculated with loop full growth from each isolate and incubated at 37 °C for 18 hours. Diluted bacterial suspensions in normal saline equal to the McFarland number 0.5, to obtain a uniform suspension with cell density of 1.5×10^8 CFU / ml were used[19].

Petri dishes prepared with Brain-Heart infusion Agar (BHA), the bacterial isolates were cultured on the media by streaking with sterile swab (for all tested bacteria), then created five wells of 6 mm diameters in agar medium utilizing sterile borer and filled with 100µl of every concentration for each extract using sterile micropipette, while the well made in the center contained the control. Then, the plates were incubated at 37°C for 24 hrs. Amikacin (5µg) was utilized as a positive control for detection the antibacterial activity, whereas *fluconazole* (25µg) was considered as a positive control for antifungal activity. Distilled water was considered as a negative control for antimicrobial activities. After incubation, the antimicrobial activity was estimated by measuring t inhibition zone diameter that expressed in (mm) around the well. The assessment was replicated three times and the mean diameter was considered[19].

3. Results and Discussion

3-1. Chemical analysis of *G. glabra*

The aqueous & ethanolic extracts of *G. glabra* contained saponins, flavonoids and glycosids, but lacked alkaloids and phenols. However, the ethanolic extract of *G. glabra* contained tannins and terpenoids, table 1.

Table 1. The results of Chemical analysis for *G. glabra*

Alcoholic extract	**Results**	**Aqueous extract**	**Results**
Saponins	+	Saponins	+
glycosies	+	glycosides	+
flavonoids	+	flavonoids	+
terpenoids	+	terpenoids	-
Phenols	-	Phenols	-
tannins	+	tannins	-
Alkaloids	-	Alkaloids	-

3-2.Antimicrobial Activities: Agar well diffusion test was done to evaluate the antimicrobial properties of aqueous and ethanolic extracts of *G.glabra* roots were against bacteria *P. aeruginosa, E. coli, S. aureus* and fungus *C. albicans*. The antimicrobial effect of extracts was assessed by calculating the zone of inhibition, table 2.

G. glabra root extracts showed various antimicrobial activities against *S. aureus, E. coli, P. aeruginosa* and *C. albicans*. Increased antimicrobial activity accompanied with increasing the concentrations of extract. All tested isolates were sensitive to both extracts at concentrations (3000 mg/ml). *S. aureus* and *E. coli* showed higher sensitivity, maximum effective inhibition for *G. glabra* aqueous extract was found against *S.aureus*, whereby the minimum inhibition was recorded against *P. aeruginosa*. Regarding *G. glabra* alcoholic extracts, the highest effect was noticed against *P. aeruginosa*, while the lowest effect was noticed on *C.albicans*. the results are in agreement with many studies investigated the antimicrobial properities in *G. glabra* roots extracts against different microbial species included Gram-negative such as *E. coli, Salmonella typhi, Salmonella paratyphi, P. aeruginos, and Shigella sonnei;* Gram-positive bacteria such as *S. aureus, Enterococcus faecalis, Streptococcus pyogenes and Bacillus subtilis*; and fungi such as *Aspergillus niger, Aspergillus fumigates.(* [15,16,14,**20,21**].

Tabel2. Inhibition zones (mm) caused by the antimicrobial activity of *G. glabra* roots extract

Microorganism	**Inhibition zone Diameter (mm)**								**Flu (25µg)**	**AK (30 µg)**
	Alcohol extract (mg/ml)				**Aqueous extract (mg/ml)**					
	500	**1000**	**2000**	**3000**	**500**	**1000**	**2000**	**3000**		
P. aeruginosa	**0**	**0**	**0**	**10**	**0**	**7**	**9**	**13**	**14**	**-**
S. aureus	**5**	**6**	**14**	**18**	**0**	**0**	**7**	**12**	**11**	**-**
E. coli	**0**	**7**	**9**	**12**	**0**	**5**	**8**	**11**	**22**	**-**
C. albicanis	**0**	**0**	**0**	**15**	**0**	**0**	**6**	**11**	**-**	**11**

AK: amikacin, Flu: fluconazole

The findings of current study agreed with those by several other authors[22,23,24] in which *G. glabra* extract showed positive inhibitory antibacterial activity against Gram-positive and Gram-negative species such as *S. aureus*, *P. aeruginosa* and *E. coli* Chabuck *et al*.[25]According to our study, *G. Glabra* exhibited activity against tested bacterial isolates with inhibition zone ranging 18-25mm; highest inhibition was seen against *E. coli* (25mm) and *P. aerogenosa* (24 mm).

In contrast, Sultana *et al*.[26] recorded antimicrobial efficacy of the *G. Glabra* extract against *S. aureus* and *E.coli* , but not *P. aeruginosa*, while, Soulef *et al*.[27] show that *G. glabra* L extract had an antibacterial effect against *S. aureus* and *P. aeruginosa*, but not the *E. coli*. Nirmala and Selvaraj[28] reported that *G. glabra* root extract did not exhibit any antimicrobial activity against *S.aureus*. Some reports have referred that *G. glabra* possesed anti-mycotic eeficacy against *C. albicans* [29,30] (Jabir, Anagha).In a study by Zhou et al[31] *antibacterial activity of G. Glabra* was intermediate against both gram negative and gram positive bacteria. A study by Karahan et al.[20] approved that methanolic root extracts of G. glabra have antimicrobial properties more effective against Gram-positive bacteria and Candida spp. than Gram-negative bacteria and concluded that environmental conditions affect the chemical and biological properties for G. glabra roots extracts. On the other hand, at last decades many studies described *G. glabra* as a potential medicinal compound for many purposes such as cure pulmonary inflammation treatment, Anticancer, antioxidant, liver disease treatment[32,33].

Our results revealed difference in sensitivity of bacteria to the *G. glabra* extract which was possibly attributed to the variation in the cell wall components, in which lipopolysaccharide(LPS) found in the outer membrane of Gram-negative bacteria is acting as a powerful permeability barrier to various compounds[34]. Extract of Licorice root includes various bioactive components and aproximalty, 40-50% of its whole dry weight components includes water-soluble bioactive compounds[25]. The antibacterial activity is mainly due to the presence of alkaloids, tannins, flavonoids, saponins, phenols and others secondary metabolites that act as a defense mechanism toward the invasion by numerous microorganisms[35]. Glycyrrhetinic acid was main bioactive triterpene glycoside found in Licorice root extracts and it has a broad range of pharmacological effects involving anti-inflammatory, anti-allergic, anti-ulcer, anti-oxidant, antidote, anti-tumor, and antiviral effects[35].Glabridin, the active constitutent of *G. glabra*, showed antimicrobial efficacy against both the Gram-positive and Gram-negative bacteria, in

addition to *C. albicans*. licochalcone A and glabridin blocked yeast-hyphal transition in *C.albican*, thus proposed as a therapeutic potential of licochalcone A and glabridin for oral infections caused by *C. albicans*[30].

The differences between the findings of this study and other studies may be due to the effect of different methods of preparation and extraction, environmental conditions in each area, that may be influence the contents of the effective chemical components in licorice community[34]. Limitations of this study mostly included lack of advanced methods for extraction, low number of bacterial isolates, lack of molecular tests and *in vivo* verification.

There are several mechanisms introduced an explanation for the medical properties of *G. glabra* such as: (i) inhibition of growth and replication of RNA viruses by Glycyrrhizin and glycyrrhizic acid for example with herpes zoster ,HIV and hepatitis C viruse. (ii) inhibition of hepatic metabolism of aldosterone and suppress 5-ßreductase by Glycyrrhizin and its metabolites. (iii) Steroid-like anti-inflammatory activity, similar to the action of hydrocortisone. (iv) inhibition of phospholipase A2 activity, an enzyme critical to numerous inflammatory processes. (v) inhibition of cyclooxygenase activity and prostaglandin formation by glycyrrhizic acid and (vi) inhibition of platelet aggregation and all factors in the inflammatory process indirectly [13,14].

Conclusion

Aqueous extract of *G. glabra* roots contained several phytochemical compounds such as saponins, flavonoids, glycosides, but lacked alkaloids, phenols, tannins and terpenoids, while the ethanolic extract contained saponins, flavonoids, glycosides tannins and terpenoids. *G. glabra* root extract exerted antimicrobial effects compared to the chemical agents.

Conflict of interest: None

References

1. Ghasemian A, Fattahi A, Shokouhi Mostafavi SK, Almarzoqi AH, Memariani M, Ben Braiek O, Yassine HM, Mostafavi NS, Ahmed MM, Mirforughi SA. Herbal medicine as an auspicious therapeutic approach for the eradication of Helicobacter pylori infection: A concise review. Journal of Cellular Physiology. 2019 Oct;234(10):16847-60.
2. Ghasemian A, Eslami M, Hasanvand F, Bozorgi H, Al-Abodi HR. Eucalyptus camaldulensis properties for use in the eradication of infections. Comparative immunology, microbiology and infectious diseases. 2019 Aug 1;65:234-7.
3. Al-Sa'ady AT (2020) Antibacterial screening for five local medicinal plants against nosocomial pathogens: Klebsiella pneumoniae and Staphylococcus epidermidis. Eurasia J Biosci 14: 553-559.
4. Fiore, C.; Eisenhut, M.; Ragazzi, E.; Zanchin, G.; Armanini, D. A history of the therapeutic use of liquorice in Europe. J. Ethnopharmacol. 2005, 99, 317–324.
5. Ram A, Singh SK, Singh VP, Kumar S, Ghosh B. Inhaled carbenoxolone prevents allergic airway inflammation and airway hyperreactivity in a mouse model of asthma.Int Arch Allergy Immunol. 2009;149(1):38-46. doi: 10.1159/000176305. Epub 2008 Nov 26. PMID: 19033731
6. Sakpa, C.L.; D.Idodia. Histopathological changes in lungs of Wistar rats treated with aqueous leaf extract of Bryophyllum pinnatum. Annals of Biomedical Sciences.2019;18(2) https://www.ajol.info/index.php/abs/article/view/188568.
7. Saeedi M, Morteza-Semnani K, Ghoreishi MR (2003) The treatment of atopic dermatitis with licorice gel. J Dermatol Treat 14:153–157.
8. Ram A, Mabalirajan U, Das M, Bhattacharya I, Dinda AK, Gangal SV, Ghosh B. Glycyrrhizin alleviates experimental allergic asthma in mice. Int Immunopharmacol. 2006 Sep;6(9):1468-77. doi: 10.1016/j.intimp.2006.04.020. Epub 2006 Jun 6. PMID: 16846841.
9. Sharma V, Agrawal RC, Pandey S (2013) Phytochemical screening and determination of antibacterial and anti-oxidant potential of Glycyrrhiza glabra root extracts. J Environ Res Dev 7(4A):1552–1558.
10. Gulati,Kavita;Pankaj Verma;Nishant Rai;Arunabha Ray. Chapter 7 - Role of nutraceuticals in respiratory and allied diseases. Nutraceuticals (Second Edition),Efficacy, Safety and Toxicity.2021, Pages 101-115. https://doi.org/10.1016/B978-0-12-821038-3.00007-0.
11. Masoomeh MJ, Kiarash G.In vitro susceptibility of Helicobacter pylori to licorice extract. Iran J Pharm Res.2007; 6:69-72.
12. De Clercq E (2000) Current lead natural products for the chemotherapy of human immunodeficiency virus (HIV) infection. Med Res Rev 20:323–349.
13. Sharma, Varsha, Akshay Katiyar, and R. C. Agrawal. Glycyrrhiza glabra: Chemistry and Pharmacological Activity.2017; chapter 4. Springer International Publishing AG 2018. https://doi.org/10.1007/978-3-319-27027-2_21.
14. Pastorino G, Cornara L, Soares S, Rodrigues F, Oliveira MBPP. Liquorice (Glycyrrhiza glabra): A phytochemical and pharmacological review. Phytother Res. 2018;32(12):2323-2339. doi:10.1002/ptr.6178.

15. Shirazi MH, Ranjbar R, Eshraghi S, Sadeghi G, Jonaidi N, Bazzaz N, Izadi M, Sadeghifard N., An Evaluation of Antibacterial Activity of Glycyrrhiza glabra Linn Extract on the Growth of Salmonella, Shigella and ETEC E. coli, J Biological Sciences, 2007; 7(5): 827-829.
16. Gupta VK, Fatima A, Faridi U, Negi AS, Shanker K, Kumar JK, Rahuja N, Luqman S, Sisodia BS, Saikia D, Darokar MP, Khanuja SP. Antimicrobial potential of Glycyrrhiza glabra roots. J Ethnopharmacol. 2008 Mar 5;116(2):377-80. doi: 10.1016/j.jep.2007.11.037. Epub 2007 Dec 4.
17. Ajagannavar,S.; Batur,H.; Shamarao,S.; Sivakomar, V.; Patil, P. and Shavanas, P. (2014). Effect of Aqueous and Alcoholic Licorice (*Glycyrrhiza Glabra*) Root Extract Against *Streptococcus Mutans* and *Lactobacillus Acidophilus* in Comparison to Chlorhexidine: An *In Vitro* StudyJournal of international oral health . V.6 NO.(4): 29–34.
18. Kodangala, Chandrashekar, Santanu Saha and Prasanna Kodangala. Phytochemical studies of aerial parts of the plant Leucas lavandulaefolia. Der Pharma Chemica, 2010, 2(5):434-437. http://derpharmachemica.com/archive.html.
19. Enciso,S.; Medina,J.; Mauricio,F.; Mauricio-Vilchez,C.; Alvitez-Temoche ,D.; Vilchez,L.; and Mayta-Tovalino,F.(2020). Antibacterial Effectiveness of Four Concentrations of the Hydroalcoholic Extract of Solanum tuberosum (Tocosh) against Streptococcus mutans ATCC 25175TM: A Comparative In Vitro Study. International Journal of Dentistry V. 2020, ID 8856382, 5 pag.
20. Karahan F., Avsar C., Ozyigit I.I., Berber I. Antimicrobial and antioxidant activities of medicinal plant Glycyrrhiza glabra var. glandulifera from different habitats. Biotechnol. Biotechnol. Equip. 2016;30:797–804. doi: 10.1080/13102818.2016.1179590.
21. Geetha R.V., Anitha R. In vitro evaluation of anti mycotic activity of ethanolic extract of Glycyrrhiza glabra. Asian J. Pharm. Clin. Res. 2013;6:205–206.
22. Karami, Z., Mirzaei, H., Emam-Djomeh, Z., Sadeghi Mahoonak, A.R. and Khomeiri, M. Effect of harvest time on antioxidant activity of *Glycyrrhiza glabra* root extract and evaluation of its antibacterial activity International Food Research Journal.2013; 20(5): 2951-2957.
23. Abkhoo, J. and Jahani, S. (2017). Antibacterial Effects of Aqueous and Ethanolic Extracts of Medicinal Plants Against Pathogenic Strains, Int J Infect.; 4(2):e42624I.
24. Saral, A., Kardil, U. And Düzgün, A. Ö. (2019).Antibacterial Activity of DMSO Extracts of Selected Plants Against Antibiotic Resistant Clinical Isolates. Erzincan University Journal of Science and Technology, 12(2), 576-584.
25. Chabuck, Z.A.G., Hadi, B.H. and Hindi, N.K.K. (2018). Evaluation of Antimicrobial Activity of Different Aquatic Extracts against Bacterial Isolates from UTI in Babylon Province, Iraq. Journal of Pure and Applied Microbiology, 12(2): 693-700.
26. Sultana, S., Haque, A., Hamid, K., Urmi, K.F. and Roy,S. (2010). Antimicrobial, cytotoxic and antioxidant activity of methanolic extract of *Glycyrrhiza glabra*. Agric. Biol. J. N. Am., 2010, 1(5): 957-960.
27. Soulef, K., Abdelouahab, Y. and Dalal, B. (2014). Effect of glycosides extract of the medicinal plant Glycyrrhiza glabra L. from the region of Mlilli (southeast of Algeria) on the growth of some human pathogenic bacteria. Journal of Scientific and Innovative Research; 3 (1): 28-34.
28. Nirmala P, Selvaraj T. Anti-inflammatory and anti-bacterial activities of Glycyrrhiza glabraL. Journal of Agricultural Technology.2011;7:815–823.
29. Jabir,S.M. (2011). The inhibitory effect of watery extracts of Glycyrrhiza Glabra roots on the growth of *Candida albicans* in comparison with antifungal antibiotics. Journal Of Wassit For Science & Medicine; ; 4(2):95-100.
30. Anagha, K., Manasi, D., Priya, L. and Meera, M. (2014). Antimicrobial activity of Yashtimadhu (Glycyrrhiza glabra L.) A Review Int.J.Curr.Microbiol.App.Sci. 3(1): 329-336.
31. Zhou, Jun-Xian; Markus Santhosh Braun , Pille Wetterauer , Bernhard Wetterauer and Michael Wink**.** Antioxidant, Cytotoxic, and Antimicrobial Activities of *Glycyrrhiza glabra* L., *Paeonia lactiflora* Pall., and *Eriobotrya japonica* (Thunb.) Lindl. Extracts. *Medicines* 2019, *6*, 43; doi:10.3390/medicines6020043. www.mdpi.com/journal/medicines.
32. Alsayari A., Muhsinah A.B., Almaghaslah D., Annadurai S., Wahab S. Pharmacological efficacy of Ginseng against respiratory tract infections. Molecules. 2021;26:4095. doi: 10.3390/molecules26134095.
33. Wahab S, Annadurai S, Abullais SS, Gotam Das, Wasim Ahmad, Md Faruque Ahmad, Geetha Kandasamy *et al*. Glycyrrhiza glabra (Licorice): A Comprehensive Review on Its Phytochemistry, Biological Activities, Clinical Evidence and Toxicology. Plants (Basel). 2021;10(12):2751. doi:10.3390/plants10122751.
34. Iqbal, Z., Hai, Z., Ping, H., Ghaffar, A., Mumtaz, M., Liaqat, L. (2017). Antioxidant and Antibacterial Activity of Organic Extracts of Roots of Glycyrrhiza Glabra Linn. Plant.5(4)2017: 68-72.

35. Celik, M. M. and Duran, N. (2019). An experimental in-vitro study to evaluate the antihelicobacter activity of Glycyrrhetinic acid. Revista Română de Medicină de Laborator.27(1)Ianuarie:63-71.