Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE

DOI: 10.47750/jptcp.2023.1103

# Detection of the bacterial activity of saponins and some mineral elements in the local aqueous extract of licorice

Zainab Hashim Mohammed[1] , Nuha Ali Hadi al-Samarrai[2] , Rabeah T . Mahmood[3]
[1,3]Department of Applied Chemistry, College of Applied Sciences, University of Samarra, Samarra, Iraq.
[2]Chemistry Department, College of Education, Samarra University.
Email: Zainab.h@uosamarra.edu.iq

**Submitted: 06 January 2023; Accepted: 05 February 2023; Published: 04 March 2023**

## ABSTRACT

The aim of the study was to detect and quantify saponins in the aqueous extract of licorice using HPLC technique. It was found that it contained the highest recorded concentration of Rhaoglucoglycyrrhizin in 335.37 µg/ml, while the lowest recorded concentration of Glycyrrhizin-20 methanoate µg /ml was 89.6. Also, the percentage of saponins in the aqueous and alcoholic extracts was 6.58% and 4.15%, respectively. The concentration of flavonoids and tannins in the aqueous extract of licorice were 18.99 and 6.28 ppm, respectively. The study also aimed to find the percentage of some major and minor metallic elements, including Fe, Na, Ca, K, Mg, Cu, Zn, Mn, and they were (1.382%, 2.04%, 5.5%, 5.38%, 4.186%, 1. 877%, 1.2%, 3.2% ) respectively. The effect of the extract containing the saponins mentioned above was studied in two bacterial species (Serratia, Klesiella Pneumoniae) using brain diffusion agar and then the added concentrations (100, 50,25,12.5)mg / Ml of the extract. The transparent zone of the bacterial growth inhibitor showed a concentration of 100 mg / ml inhibitory zone for Klesiella Pneumoniae and 27 mm for Serratia .

**Keywords:** color reagents, aqueous extract, saponins, elements , Serratia, Klesiella Pneumoniae.

## INTRODUCTION

Licorice herb (Glycyrrhiza glabra), which is used to treat many diseases, as licorice roots are the main commercial important source of drugs and medicines because they contain active substances such as (Glycyrrhiza) derived from the Greek word glykos meaning sweet and rhiza meaning root) ie sweet root [(1)]. A study in Japan showed that this substance is effective in treating chronic hepatitis and cirrhosis of the liver, as it was found that 75% of patients with liver failure had an increase in their liver activity after using a dose of 240 mg per day for only one month[( 2)]. It has been observed that the herb has an effective role in activating the aldosterone-angiotensin-renin system, as it is believed that the soapy substances present in the licorice herb activate the effectiveness of aldosteron through its association with the receptors of the mineral cortex in the kidneys [(3)]. The study showed the use of licorice in the treatment of injuries Bacterial, viral and fungal, and that some of the compounds derived from it are of great importance in the treatment of some cancerous diseases. Glabridin acts as an anti-division of breast cells ex vivo, in addition to its use in the treatment of gastrointestinal ulcers. It increases the secretion of the mucous glands of the system that helps treat ulcers [(4)], and research has proven that the plant possesses an oxidant-anti-oxidant activity.[(5,6 )]

The active substance in licorice is glycerite, and it has been proven that licorice contains sugary substances and mineral salts, the most important of which are potassium, calcium, magnesium, phosphorous, and soapy substances that cause foam when pouring its juice, and it also contains volatile oil. In the year (1955-1960) a steroid compound was separated and called glycerrhysic acid from the roots of the licorice plant. [(7)]

Saponins are bioorganic compounds that are found in many plants and foods. These compounds are part of the group of phytosterols, saponins and are nutrients that have many health benefits such as lowering cholesterol, helping to control blood sugar levels and preventing the onset of cancer. It has anti-inflammatory, antioxidant, anticancer, immunostimulant, cytotoxic and antimicrobial properties [8].

Saponins are powerful antioxidants that protect cells from free radicals, and help prevent changes in DNA that can lead to diseases such as cancer. In addition, its antioxidant power also reduces the formation of atherosclerotic plaques in the blood vessels, which prevents problems such as heart attack and stroke. Saponins reduce cholesterol levels in the blood and liver, as they reduce the absorption of cholesterol from food in the intestine. In addition, they increase the excretion of cholesterol in the stool by increasing the elimination of bile acids [9,10].

## MATERIALS AND METHODS

- Preparation of the aqueous extract:
  10 gm of dry ground licorice root was dissolved in 200 ml of distilled water for 24 hours with continuous stirring, then filtered. The extract concentrated and kept in refrigeration until use [11].
- Preparing the inductive reagents:

1. Detection of alkaloid: Alkaloid was detected by adding drops of Mayer's reagent to 1 ml of the extract to form a white to pink color [12]
2. Detection of sterols: adding some drops of sulfuric acid to 1 ml of the extract to give a red color [13]
3. Detection of saponites: Mix 1 ml with (1 $cm^3$) of (1% mercury chloride $HgCl_2$) to form a white precipitate [14]
4. Detection of turbines: Detection was carried out using turm-hill detection [15].
5. Detection of tannins and phenols using glacial acetic acid [16]
6. Detection of glycosides: using sodium hydroxide solution [17]
7. Detection of flavonoids using ferric chloride [18]

- Quantitative determination of saponins:
  according to the method used by [19]) with aqueous and alcoholic solutions only [19]
- Quantitative determination of flavonoids: An estimated weight of 25 g using a mixture of methanol and chlorform only [20]
- Quantitative determination of tannin: 10 gm of licorice root [21]
- Quantitative and qualitative determination of saponins in aqueous extract using HPLC only [22]
- Determination of mineral elements: some mineral elements were estimated by atomic abs Chemicals and instruments.
- Antibacterial activity
  The antibacterial potential of the prepared Samples [23] was investigated against Gram's negative and Gram's positive bacterial strains using agar well diffusion assay [24, 25]. About 20mL of on Muller–Hinton (MH) agar was aseptically poured into sterile Petri dishes. The bacterial species were collected from their stock cultures using a sterile wire loop [25]. After culturing the organisms, 6 mm-diameter wells were bored on the agar plates using of a sterile tip. Into the bored wells, different concentrations of aqueous extract of licorice were used. The cultured plates containing aqueous extract of licorice and the test organisms were incubated overnight at 37°C before measuring and recording the average the zones of inhibition diameter [26,27].
- Statistical analysis.
  Data were statically analysis using Graphpad prism program [28]. Data are represented as mean ± SD of three experiments. Indicate statistically significant difference at $p<0.05$ [29,30].

## RESULTS AND DISCUSSION

It is evident from table (1) that the aqueous extract of licorice contains alkaloids, saponins, tannins and glycosides, which are secondary metabolites.

**TABLE 1:** Phytochemical screening of the aqueous extract of licorice

| **Phytochemical** | **Test** | **Result** |
|---|---|---|
| **Alkaloids** | Alkaloids | + |
| **Phytosterol** | Salkowski | - |
| | Libermann-Burchard | |
| **Saponins** | $HgCl_2$ | + |
| **Terpenoids** | Trim-Hill | - |
| **phenolic compounds and tannins** | Ferric chloride | + |
| **Glycosides** | Alkaline reag. | + |

(+) positive detection
(-) negative detection
It was found through the current study that the percentage of saponins in the aqueous extract was 6.58%, while that of the alcoholic extract was 4.15%. It was found through the current study that the concentration of flavonoids is 18.99 ppm, while the concentration of tannin is 6.23 ppm

**TABLE 2:** shows the percentage of saponins isolated from licorice using different solvents

| **Saponins** | **Solvents** | **% saponins** |
|---|---|---|
| | Aqueous | 6.58% |
| | Alcoholic | 4.15% |

**TABLE 3**: Concentration of flavonoids and tannins isolated from licorice

| **Licorice** | **Extracts** | **Con. Ppm** |
|---|---|---|
| | Flavonoids | 18.99 |
| | Tannin | 6.28 |

Figure (1) showed the standard form of saponins separated by HPLC using a separation column C18,4.6mm x150mm,5µ.

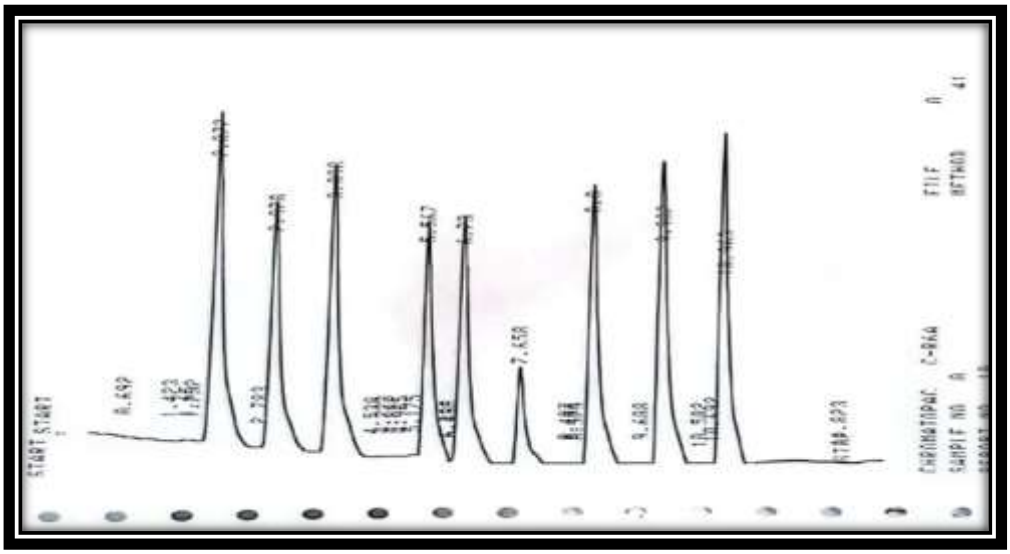


**FIGURE 1**: The standard form of saponins.

**TABLE 4:** shows the standard saponins, the retention time and the separated beam area by HPLC device.

| Seq | Sapoinine | R.t | Aare | Con.µg/ml |
|---|---|---|---|---|
| | 30-yrrhydroxyglycyrrhizin | 2.07 | 69104 | 50 µg/ml |
| | 24-hydroxyglucoglycrrhizin | 2.97 | 94627 | 50 µg/ml |
| | Glycyrrhizin-20 methanoate | 3.89 | 59352 | 50 µg/ml |
| | Rhaoglycyrrhizin | 5.65 | 49002 | 50 µg/ml |
| | 11-deoxorhaoglycyrrhizin | 6.73 | 51061 | 50 µg/ml |
| | Rhaoglucoglycyrrhizin | 7.65 | 20004 | 25 µg/ml |
| | Rhaogalactoglycyrrhizin | 8.8 | 60053 | 50 µg/ml |
| | 11-deoxo-20- & glycyrrhizin | 9.90 | 65399 | 50 µg/ml |
| | Glucoliquiritin apioside | 10.96 | 72510 | 50 µg/ml |

Figure (2) showed the two saponins separated from licorice, the time of detention and the area of the bundle separated by the HPLC using a separation column C18,4.6mm x150mm,5µ.

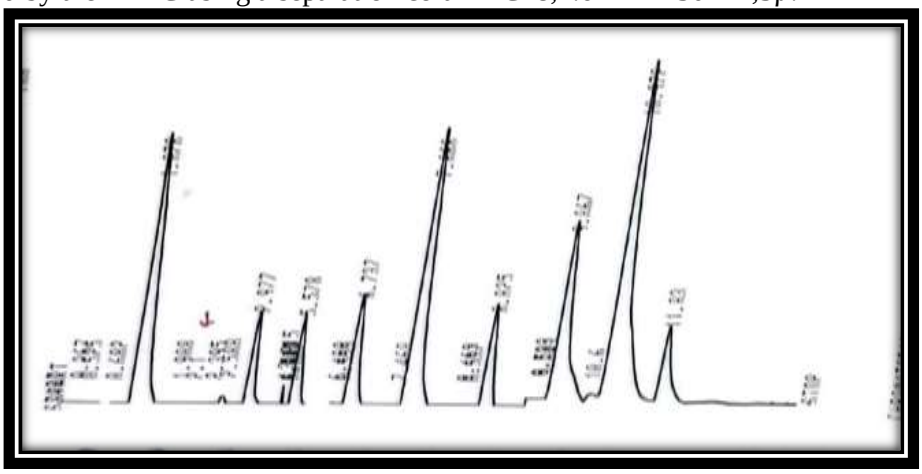


**FIGURE 2**: saponins separated from aqueous extract of licorice root.

**TABLE 5:** The separated saponin concentration, retention time, and top area of the aqueous extract of licorice.

| Seq | Sapoinine | R.t | Eare | Con. µg /ml |
|---|---|---|---|---|
| | 30-yrrhydroxyglycyrrhizin | 2.1 | 5000 | 180.08 µg |
| | 24-hydroxyglucoglycrrhizin | 2.97 | 42638 | 214.79 µg |
| | Glycyrrhizin-20 methanoate | 3.89 | 21273 | 89.6 µg |
| | Rhaoglycyrrhizin | 5.65 | 36740 | 187.44 µg |
| | 11-deoxorhaoglycyrrhizin | 6.73 | 37010 | 181.2 µg |
| | Rhaoglucoglycyrrhizin | 7.65 | 56860 | 335.37 µg |
| | Rhaogalactoglycyrrhizin | 8.8 | 28733 | 119.6 µg |
| | 11-deoxo-20- & glycyrrhizin | 9.90 | 61096 | 233.5 µg |
| | Glucoliquiritin apioside | 10.96 | 92637 | 319.39 µg |

**TABLE 6**: shows the percentage of some mineral elements in licorice.

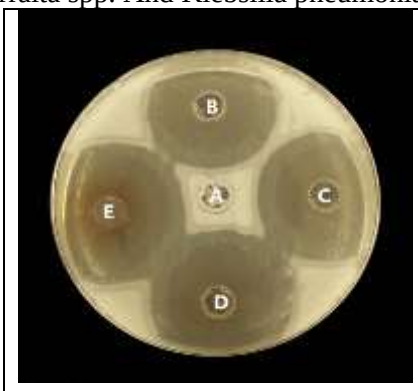
| Minerals | % |
|---|---|
| Fe | 1. 3824 |
| Na | 2.04 |
| Ca | 5.5 |
| K | 5.38 |
| Mg | 4. 186 |
| Cu | 1. 877 |
| Zn | 1.2 |
| Mn | 3.2 |

Result of antibacterial activity with different concentration shown by the figures below all details explained by table (6)

**TABLE 6:** Explain the antibacterial activity of aqueous extract of licorice

| Antibacterial analysis (Inhibition Zone) | | | | | | |
|---|---|---|---|---|---|---|
| sample | | A | B | C | D | E |
| Serratia | aqueous extract of licorice | 6 | 24 | 25 | 26 | 27 |
| Klesiella Pneumoniae | | 6 | 19 | 21 | 23 | 26 |

Figure (3,4) shows the antibacterial activity Antibacterial activity of aqueous extract of licorice against Serraita spp. And Klebsilla pneumoniae.



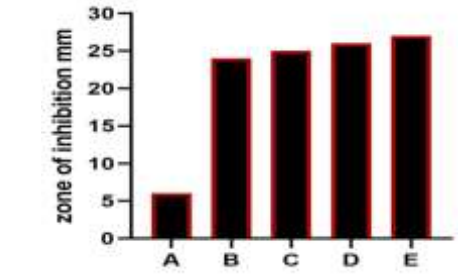
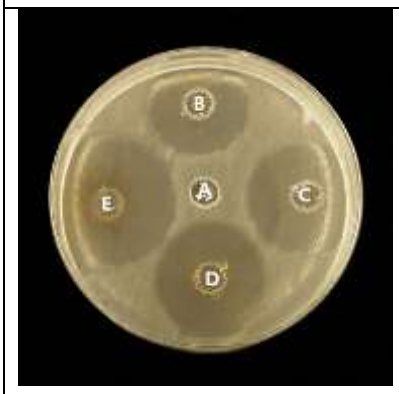




**FIGURE. 3:** Antibacterial activity of aqueous extract of licorice against Serraita. A, control negative . B, 12.5%. C,25 %. D,50 %. E, 100 %



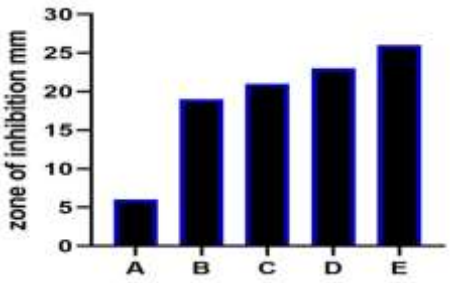




**FIGURE 4**: Antibacterial activity of aqueous extract of licorice against Klebsilla pneumoniae. A, control negative . B, 12.5%. C,25 %. D,50 %. E, 100 %

## DISCUSSION

The results of the current study are consistent with what was found by Sahi and Khudair [31,32], where the latter found that the aqueous extract of licorice contains saponins and flavonoids, while the latter noted that the aqueous extract of licorice did not contain alkaloids. The results of the current study do not agree with Hamza [33], where the latter found that the alcoholic extract contains flavonoids, saponins and resins, while it does not contain alcoedes. Also, the results of the current study agree with Ayoub [34], where he indicated that the aqueous extract contains flavonoids, saponins, glycosides and tannin.

Also, the results of the current study are consistent with the result of Kumar et al. [35] who found that the aqueous extract contains saponins, tannins and flavonoids. While it does not contain alkaloids.

It was found through the current study that the percentage of saponins in the aqueous extract was 6.58%, while that of the alcoholic extract was 4.15%. The results of the current study agree with Al-Ajimi [36], where the latter found that the percentage of saponins in licorice is (4-24)% in the aqueous extract.

The current study agrees with what was reached by Newall et al [37]. The results of the current study do not agree with what was found by Mohammed [38], where the latter found that the concentration of flavonoids in the aqueous extract is 2.3ppm and the reason for this may be due to the type of environment in which the plant was grown. The results of the current study are in agreement with Roth [39], where the latter found that the aqueous extract contained flavonoids and phenolic compounds. The roots of licorice contain multiple compounds, the most important of which is saponin, known as glycerin It has a sweet taste that is more than fifty times more sugary than the sugar produced from sugar cane and bee honey. It is found in the form of calcium and potassium salts at a rate of 15%-2, and when hydrolyzed or enzymatically decomposed, a compound known as and Liquiritin Chalcones ,Flavans, Flavones, Glabridin [40]. The roots contain flavonoids that give the roots a bright yellow color, gums and 15% Resinous Oils and other substances, including 2-6% asparagine, mannitol and atropine [41]. Atropine, coumarins, choline, betaine and hormone-related substances Progestogen and steroids similar to adrenocorticotropic hormone [42]. From the quantitative estimation of the aqueous extract of licorice, it was found that it contained many saponins. It contains the highest 30-yrrhydroxyglycyrrhizin,24-hydroxyglucoglycerrhizin, Glycyrrhizin-20 methanoate, Rhaoglycyrrhizin, 11-deoxorhaoglycyrrhizin, Rhaoglucoglycyrrhizin, quirkyrhaoglycyrrhizin 180, , 214.79, 89.6, 187.44, 181.2, 335.37, 119.6, 233.5, 319.39) in µg /ml units, respectively .The highest recorded concentration was Rhaoglucoglycyrrhizin 335.37 µg /ml, while the record concentration of Glycyrrhizin-20 methanoate was 89.6 µg /ml. The results of the current study are in agreement with the findings of Andrisano et al. [43] that licorice contains the soapy compounds glycyrrhetic acid and 24-hydroxy-18 beta-glycyrrhetic acid. Finally, the alcoholic extract of licorice contains glycyrrhizin using HPLC.

The results of the current study agree with Mahmoud et al. [38], where the latter found that licorice contains Glycyrrhizin and Glycyrrhetinic acid, Glabridin. The results of the study agree with what was found by Ayoub [34], where the latter found that licorice contains major and minor essential mineral elements. Through the current study, it was found that the percentage of calcium is 5.5%, potassium is 5.38%, and magnesium is 4.186%, while Ayoub found that the percentage of potassium is 2.9%, while calcium is 2.1% and magnesium 1.3%[34].

## CONCLUSION

The method of separation of saponins aqueous extract from licorice by HPLC technique, the concentration of saponins in the licorice extract was found to be close after comparing with different concentrations of the standard material . It was found that it contains some metallic elements, it has the ability to inhibition some types of bacteria that infect the respiratory system.

## REFERENCES


1. Ploeger B , Mensinga T, Sips A , et al The pharmacokinetics of glycyrrhizic acid evaluated by phtsiologically based pharmacokinetic modeling . Drug Metab Rev.2001 ; 33 : 125 – 147 .
2. Fujioka T . Kondou T .Fukuhara A . etal .Efficacy of glycyrrhizin suppository for the treatment of chronic hepatitis C: apilot study . Hepatol Res 2003 ; 26 (1) : 10 –14.
3. Fukai, T., Satoh, K., Nomura, T. and Sakagami, H.(2003). Preliminary evaluation of antinephritis and radical scavenging activities of glabridin from glycyrrhiza glabra. Fitoterapia. 74(7–8):624–629.
4. Kamej . J , Nakamura R , Ichiki H etal Tutitussive principles of glycyrrizae radix amain component of the kampo preparations bakumondo – to ( Mai - men –dongtang ). Eurj pharmacol 2003 ; 469 ( 1-3 ) : 159 -163 .
5. Tamir , S. ; Eizenberg , M. ; Somjen , D. ; Stem , N. ; Shelach , R. ; Kaya , A. and Vaya , J. (2000). Estrogenic and anti proliferative properties of glabridin from licorice in human breast cancer cell. Cancer. Res. 60 : 5704-5709.
6. Nishino, H.; Kitagawa, K. and lwashima, A. (1984) Antitumor- promoting activity of glycyrrhetic acid in mouse skin tumor formation induced by 7, 12- dim-. Ethy. benza [a] anthracene plus teleocidin carcinogen. Esis, 11: 1529- 1230.
7. Isbrucker, R. A. and Burdock, G. A.(2006).Risk and safety assessment on the consumption of licorice root Glycyrrhiza sp., its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. Regul. Toxicol. Pharmacol. 46:167–192
8. Edward, G. R., Bene, D. R., Lindsay, R. S. and Seckl, J. R.(1996).11 betahydroxysteroid dehydrogenase: key enzymes in determining tissue–specific glucocorticoid effects. Steroids. 61:263–269.
9. Mori,K.;Sakai,H.;Suzuki,S.Augai,K.;Akustsu,Y.;Ishi kawa,M.; Seino,Y.Ishada,N. and Uchaida,T.(1989).Effect ofglycyrrhizin on Hemophilia Patient with HIV ifection.J.Exp.Med.128:25-36.

10. Orlando,J.M. and Eva,T.(2003).Human Chromosome.4th ed. New York. USA.87-130.
11. Daher-Hjaij, N.Y., B.S. Khaled, M. El-Elan, H. Kuhayl, A. El-Menoufi and M. Hasan. 2018. Effect of the Aqueous and Alcoholic Extracts of Rosemary (Rosmarinus officinalis L.) to Control the Bee Varoa (Varroa destructor Oud.). Arab Journal of Plant Protection, 36(1): 27-31.
12. Kokate, C.K.; Purohit, A.P. and Gokhale, S.B. Pharmacognosy. Nirali Prakashan 2009; 6:16-17.
13. Gibbs, R.D. Chemotaxonomy of Flowering Plants. Montreal and London, McGill Queen's University Press,. 1974.
14. Mahmoud, M. J. Chemistry of medicinal plants. Printed in Anwar Dijla, Bagdad, raq 2008:13-16.
15. Rashant ,T.; Bimlesh, K., Mandeep, K.; Gurpreet,K. and Harleen, K. Phytochemical screening and Extraction: A Review.Int. Pharm. Sci. 2011;1(1): 98-106.
16. Kokate, C.K.;Purohit, A.P. and Gokhale, S.B. "Pharmacognosy"17th ED , Nirali Prakashan 2009:p. 99, 231, 185.
17. Harborne, J.B. Phytochemical methods. 2nd ED, New York, USA.Chapman and hall, (1985).
18. Sofowara, A. Medicinal plants and Traditional medicine in Africa. Ibadan, Nigeria ,Spectrum Books Ltd.1993. p. 289.
19. Ezeabara,chinelo A ; Okeke, C.U; Aziagba ,Bibian .O. et al. Determination of Saponin Co tent of Various parts of Six Citrus Species. International R esearch Journal of pure and Applied Chemistry. 4(1):137-143.2014.
20. AL-Samarra ,Nuha Ali Hadi . Estimate of the flavonoids in the Leavesof BayLaurel Studyingtheir Bio-activity . SSN: 1813 –1662 (Print)E-ISSN: 2415 –1726 (On Line). Tikrit Journal of Pure Sciences .22 (7) 2017.
21. Jabbar ,Maryam Hadi . Al-Ghanimi Ali Abdul Kadhim . Kadhim Najeh Hashem . Extraction and partial Purification of Proanthocyanidins from Grape (Vitis vinifera ) and Date (Phoenix dactylifera) seeds and determination some of their Biological activities. Master of Science in Biology. Kerbala University 2006.
22. Wu Xuejin , Jia Lingyan ,Wu Jiafan et al. Simultaneous Determination and Quantification of Triterpene Saponins from Camellia sinensis Seeds Using UPLC-PDA-QTOF-MS/MS. Molecules 2019, 24, 3794; doi:10.3390/molecules24203794.
23. Bahjat, H. H., Ismail, R. A., Sulaiman, G. M., and Jabir, M. S. (2021). Magnetic field-assisted laser ablation of titanium dioxide nanoparticles in water for anti-bacterial applications. Journal of Inorganic and Organometallic Polymers and Materials, 1-8.]
24. Khashan, K. S., Abdulameer, F. A., Jabir, M. S., Hadi, A. A., and Sulaiman, G. M. (2020). Anticancer activity and toxicity of carbon nanoparticles produced by pulsed laser ablation of graphite in water. Advances in Natural Sciences: Nanoscience and Nanotechnology, 11(3), 035010.
25. Khashan, K. S., Badr, B. A., Sulaiman, G. M., Jabir, M. S., and Hussain, S. A. (2021, March).Antibacterial activity of Zinc Oxide nanostructured materials synthesis by laser ablation method. In Journal of Physics: Conference Series (Vol. 1795, No. 1, p. 012040),
26. Jihad, M. A., Noori, F., Jabir, M. S., Albukhaty, S., AlMalki, F. A., and Alyamani, A. A. (2021). Polyethylene Glycol Functionalized Graphene Oxide Nanoparticles Loaded with Nigella sativa Extract: A Smart Antibacterial Therapeutic Drug Delivery System. Molecules, 26(11), 3067.
27. Mohammed, M. K., Mohammad, M. R., Jabir, M. S., and Ahmed, D. S. (2020, March). Functionalization, characterization, and antibacterial activity of single wall and multi wall carbon nanotubes. In IOP Conference Series: Materials Science and Engineering (Vol. 757, No. 1, p. 012028). IOP Publishing.
28. Ali, I. H., Jabir, M. S., Al-Shmgani, H. S., Sulaiman, G. M., and Sadoon, A. H. (2018, May). Pathological And immunological study on infection with escherichia coli in ale balb/c mice. In Journal of Physics: Conference Series (Vol. 1003, No. 1, p. 012009). IOP Publishing.
29. Younus, A., Al-Ahmer, S., and Jabir, M. (2019). Evaluation of some immunological markers in children with bacterial meningitis caused by Streptococcus pneumoniae. Research Journal of Biotechnology, 14, 131-133.
30. Jabir, Majid S., Taha M. Rashid, Uday M. Nayef, Salim Albukhaty, Faizah A. AlMalki, Jawaher Albaqami, Amal A. AlYamani, Zainab J. Taqi, and Ghassan M. Sulaiman. "Inhibition of Staphylococcus aureus α-Hemolysin Production Using Nanocurcumin Capped Au@ ZnO Nanocomposite." Bioinorganic Chemistry and Applications 2022 (2022).
31. Ifesan, B. O. T.; O. S. Ijarotimi, and O.F. Osundahuns. (2006). Evaluation of the antioxidant activity of Ocimum sp. J. Food Technol., 4(4):318.
32. Sahi ,Nibras Mohamed ; Khudair, Hadi Mezal . Evaluation of culture
33. compounds isolated from leaves and roots of licorice plant in some aspects
34. of biological exclusion of Culex pipiens mosquitoes. Journal of Babylon
35. University for Pure and Applied Sciences.1(23)2015.
36. Hamza Doaa abdul Kareem . Effect of ethanol extract of root Glycyrrhiza glabra plant in liver enzymes levels and some histological and hormonical and Genetic standards in males white rats Induced Mitomycin-C. Master in Biology – Zoology. College of Education Pure Science ofKarbala University. 2015.

37. Ayoub Afaf Abdel Rahman. Study of the Active Ingredient of Licorice Plant and Applications . Journal of engineering and technology . DOI: http://dx.doi.org/10.30684/etj.36.3C.8 .3(36)2018.
38. Kumar Avnish, Asthana Monika, Singh Preeti ,et al . Antioxidant and activity of root extracts of Licorice antibacterial ( Glycyrrhiza glabra ). International Journal of Minor Fruits, Medicinal and Aromatic Plants. Vol. 6 (1) : 01- 12, June 2020.
39. Al-Ajaimi, Thamer Abdullah Zazan. The effect of gibberellin GA3 and some nutrients on the production of glycyrrhizin and some Other components in the licorice plant Glycyrrhiza glabra. Ph.D. thesis, College of Agriculture, University of-Iraq-28-Baghdad.2005.
40. Newall, C.A.; Anderson, L.A. and Phillipson, J.D. (Editors)(1996). Herbal Medicines The Pharmaceutical Press, London, pp:183- 186.
41. Mahmoud Istibraq Ezz El-Din, Hamid Atyaf Saeed, and Al-Ibrahimi Thamer Abdel- Shaheed Mohsen. The effect of some petroleum ether extracts of rosemary and licorice plants on the growth of Some types of plant pathogenic fungi. Journal of Mustansiriya Science.4(23)2012.
42. Roth,K; Licorice SpiralsChemie in unserer zet.38,202-207.2004.
43. Wang, Z.Y., Athar, M. and Bichers, D. R.(2000). Licorice in foods and herbal drugs: chemistry, pharmacology, toxicology and uses. In: Mazza, G. and Oomah, B. D.(editors). Herbs, botanicals and teas. Technomic Publishing Co Inc., Lancaster, PP:321–353.
44. Chin, Y. W., Jung , H. A. and Liu, Y.(2007). Anti-oxidant constituents of the roots and stolons of licorice (G.glabra). J. Agric. Food Chem.55(12):4691-4697.
45. Isbrucker, R. A. and Burdock, G. A.(2006).Risk and safety assessment on the consumption of licorice root Glycyrrhiza sp., its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin Regul. Toxicol. Pharmacol. 46:167–192.
46. Andersen Øyvind, M., Markham, Kenneth R. (2006). Flavonoid chemistry biochemistry, and applications. CRC Press Taylor & Francis Group.