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ANTIBACTERIAL ACTIVITY OF *QUERCUS INFECTORIA* GALL EXTRACTS AGAINST MULTIDRUG RESISTANT BACTERIA

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Abstract

To investigate the antimicrobial action, isolation of dried *Quercus infectoria* galls (QI) using extraction procedure were conducted with two different solvents methanol and acetone. The bioactive constituents in QI galls extracts have been identified by using HPLC and FT-IR technique. The antibacterial activity of QI was assessed using the agar-well distribution procedure, toward pathogens medically separated *Staphylococcus aureus, Pseudomonus aeruginosa* and *E.coli*, inhibition zone diameter amount was measured. Ten sharp peaks were noticed from the results of HPLC analysis, indicating the presence of bioactive compounds. Also the result demonstrated, rich sources of Gallic acids and Tannic acids in the *Q. infectoria*, act as a strong astringent. Moreover, by FT-IR technique the main important functional groups were reported. The FTIR spectrum was confirmed that the QI presence common functional groups of alkanes, alkenes, phenols, nitro compounds, aliphatic fluoro compounds, as well as hydrogen bonded alcohols. This research suggest that *Q. infectoria* galls-extract can could be used as complementary and alternate medicine in the in the therapy of infectious diseases, induced by the range of bacteria tested.

***Key words*:** Gall of *Quercus*, HPLC analysis, FTIR, antibacterial activity.

Introduction

Pharmaceutical herbs were utilized in Conventional therapy at various parts in the world, specifically in remote regions. The World Organization for health approximated that 80% of the Third-world population Nations rely on conventional main healthcare drugs. The fundamental parts obtained from therapeutic herbs possess the capacity to create a broad range of bioactive chemicals therefore, fresh crops have to be identified for a wide range of clinically precious materials. *Quercus infectoria* galls (QI) is a short tree of the Fagaceae family that is commonly spread in Greece, Iran and minor Asia, that is famous as an oak tree as well. The QI galls appear on this tree's young stems as a result of an assault by *Adleria's* female *gasp-wasp*. The seeds are formation with *Adleria gallae-tinctoria* and *Cynipsgallae tinctoria*. The galls are spherical in form with an internal color of green yellow and heavily aromatic odor.

The *Q. infectoria* of galls is used widely as a natural product because it has been recorded contain high quantities of biologically active ingredients like tannins, ellagic acid, gallic acid, syringic acid, β-sitosterol, amentoflavone, hexagalloylglucose, hexamethyl ether, methyl oleanate, methyl betulate and isocryptometrin. Tannin (50-70%), gallic acid (2-4%) and ellagic acid are the principal ingredients in the galls. Earlier study discovered that nutgall ethanol processing comprises tannins, flavonoids and steroidal compounds. In addition, galls are also commonly used for the post birth procedures hemorrhage, diarrhea and skin cancer therapy. In Asian, the hydrous extract of QI galls has been used as a traditional treatment for the therapy of inflamed tonsils for periods, while immediate implementation of heated QI had been used to cure skin swelling and inflammation.

Moreover, galls of the *Q. infectoria* have been pharmacologically decrypted as anti-diabetic, antipyretic, astringent, anti-tremor, local aesthetic, anti-Parkinson, anti-osteoporotic, anti-osteoporotic, antifungal, antibacterial, antiviral, anti-inflammatory and wound-healing. The *Q. infectoria* of gall-extracts versus *Anopheles* stephensi were originally recorded. Therefore the scientists paid special attention to the therapeutic need for drugs obtained

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from medicinal herbs to a set of variables, such as protection, efficacy and financial sustainability. Thus, the research targeted at evaluating acetone, methanol the *Q. infectoria* extract galls have been explored and their impact on the antibacterial.

Materials and Methods

Materials

All the substances used for this study were technical and acquired from the University-Iraq region of Erbil Health Technical College. The materials and ordinary solutions were produced utilizing distilled water.

Collection of the product

Dried QI of the galls was obtained from Erbil, Kurdistan herbal shop. Depending on their attributes, the galls were recognized and washed entirely to avoid foreign products. Then, pot and rolling pin smashed the sample into tiny pieces and crushed it in an electric grinding machine. After that, the dry powders were held in enclosed container until the day of extraction to avoid the effect of moisture at room temperature.

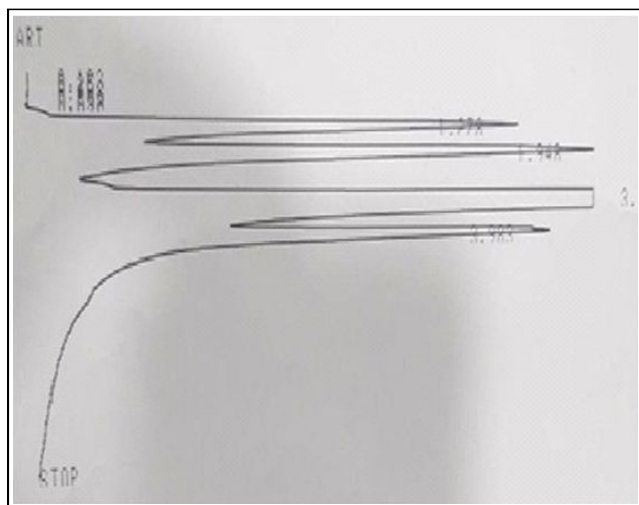

Fig. 1: HPLC chromatogram of methanol extract *QI*

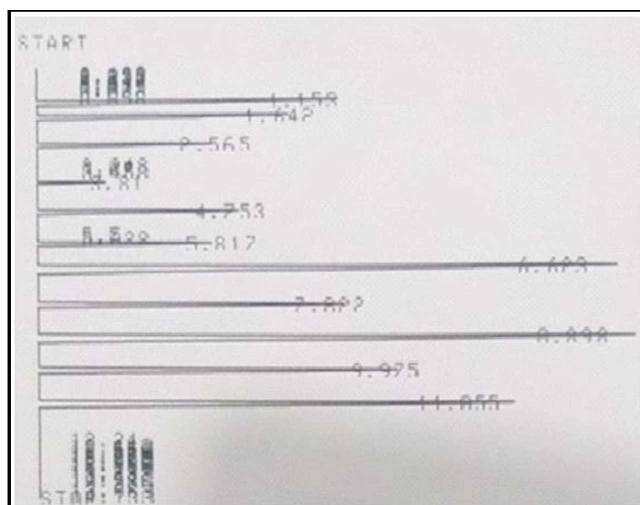

Fig. 2: HPLC chromatogram of acetone extract *QI*

Preparation the extraction procedure

This operation was carried out according to, Gall powder (100g) was immersed in 500ml methanol at room temperature with 25°C for 24hours. The obtained solution was passed through a filter paper Whatman (No. 1) and followed the entire system using the remaining 300ml methanol residue. The filtrates were merged with a rotary evaporator and concentrated. Finally, the later pellet was processed below a warm air dryer to obtain the dried crude methanol extract galls of QI. The powder shape was located in a refrigerator in covered glass bottles at 4°C till the day of HPLC and FT-IR analysis. The whole process has been repeated for the acetone solvent as well.

Procedure of HPLC analysis

Below the ideal situation Column, the primary phenolic chemical was isolated by Fast Liquid Chromatographic (FLC) column: Phenomenex C-18,3µm (50×2.0mm I.D) column, Mobile phase: fluid gradation, liquid A 0.1% phosphoric acid: Solvent B was (6:3:1, v/v) of acetonitrile: methanol: 0.1% phosphoric acid, linear gradient from 0% B to 100% B for 10min. 1.0ml/min flow rate, detection: 280UV and each standard was 25µg/ml.

Equipment

The removal resulted on Shimadzu 10AV-LC liquid chromatography fitted with the parallel supply engine design LC-10A Shimadzu, the eluted peaks were observed by the spectrophotometer UV-Vis 10A-SPD.

Extraction

0.5g of leaves powder was dissolved in 20ml hexane to eliminate the fat layer, as well as the organic layer

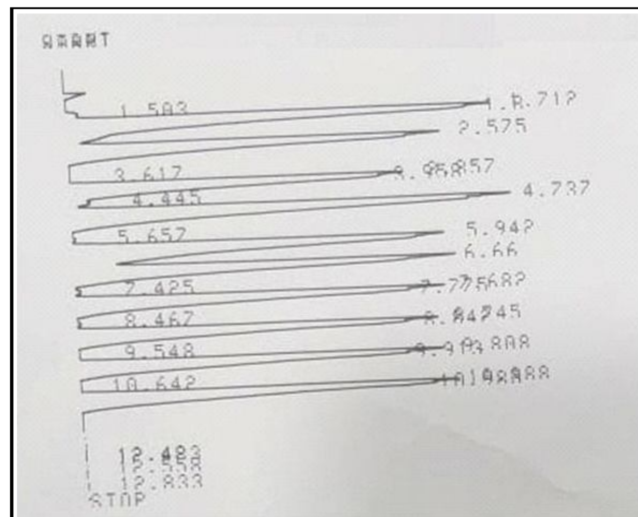

Fig. 3: HPLC chromatogram of standard compounds.

Table 1: The Concentration (µg/ml) of bioactive compounds extracts from *Q.infectoria* as calculated by HPLC.

Seq.	Compounds	Retention Time (min)	Area (µvolt)	Concentration of standard	Methanolic Conc.	Methanolic Conc. (X 20=)	Acetone Conc.	Acetone Conc. (X 20=)
1	Gallic acid	1.80	60841	6.6622	4.910	98.2	7.90	158
2	Protocatecunic acid	2.57	80374	8.801	7.819	156.38	6.402	128.04
3	catechins	3.95	44107	4.829	4.552	91.04	4.874	97.48
4	Tannin	4.73	110658	12.117	4.641	92.82	6.950	139
5	Caffeic acid	5.94	93542	10.2429	6.021	120.42	6.430	128.6
6	Chlorogenic acid	6.66	91596	10.0298	14.144	282.88	14.036	284.94
7	Vanillic acid	7.77	57696	6.317	18.952	379.04	9.132	182.64
8	Coumaric acid	8.84	56640	6.202	16.868	326.88	14.247	284.94
9	Luteolin	9.91	56774	6.216	18.344	366.88	10.154	203.08
10	Quercetin	10.98	62286	6.820	15.912	318.24	12.004	240.08
	Total			71.5747	107.253	2134.58	84.229	1688.8

was dissolved by 80:20ml (methanol: water), The extract was ultra-sonication (Branson sonifier, USA) for a duration of 25min at 25°C at 60 percent duty times. After that, precisely was centrifuged for 15min, at 7,500 rpm. The transparent centrifugation of each instance was subjected to coal until evaporation under vacuum to get a hold of dyes (Buchi Rota vapor Re Type). Dry parts were once again held by overtaxing in 1.0ml of methanol and HPLC. The combination was moved through 2.5µm filter paper and kept for further investigation at 4°C. Finally, under optimum conditions, 20ml of the sample was applied to HPLC system.

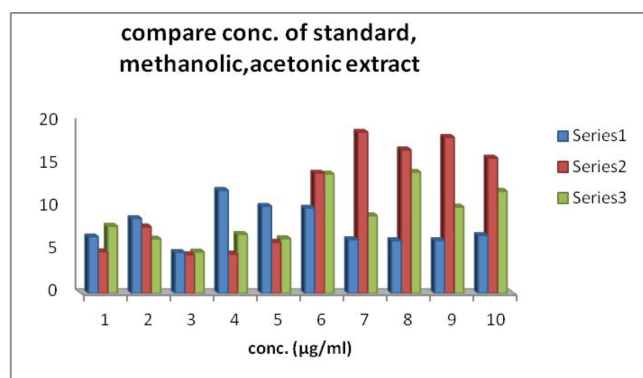

Fig. 4: Comparison the contents of standard, methanol and acetone extract of *QI*.
Series 1 = Standard, Series 2 = Methanol, Series 3 = Acetone.

Screening for the extract by disk diffusion technique

The experiment on disk spreading was carried out using the regular method. The collection of each microbial bacterial species (*S. aureus, E.coli* and *Pseu. aeruginosa*) inoculums was swabbed across the entire Mueller-Hinton agar surface. (6-mm) filter paper disks (Schleicher and Schuell) were placed on MHA surface under aseptic conditions and crude ethanol extracts or essential oils were mixed instantly with DMSO one by one volume applied to the 20µl volume disks. A 10% DMSO 20-µl aliquot was also added as an adverse control to a sterile paper disk. The dishes were left for duration of 15min at room temperature to permit excess pre-diffusion between the extracts at 37°C for 24h. before incubation. The diameters of the inhibition zones have been calculated. The experiments have been carried out in duplicates so as to eliminate the errors.

Table 2: FT-IR peak values of acetonic extract of *Quercus infectoria*.

No.	Peak (Wave number cm^{-1})	Functional group assignment	Group frequency
1.	746	C-H bend	Alkene
2.	858	C-H bend	Alkene
3.	1178	C-O stretching	Carboxylic acid, ether, ester, alcohol
4.	1321	$C-NO_2$	Nitro compounds
5.	1448	C-H	Alkane
6.	1508	NH bends	Amines
7.	1616	NH bend	Amines
8.	1735	C-H bend	Phenyl group substitution
9.	3155	OH	Hydrogen bonded alcohols, phenols
10.	3230	OH	Hydrogen bonded alcohols, phenols
11.	3321	OH	Hydrogen bonded alcohols, phenols
12.	3377	OH	Hydrogen bonded alcohols, phenols

Results and Discussion

High performance liquid chromatography was carried out in two different types of solvent methanolic and acetonic dried galls concentrate of *Q. infectoria*. The HPLC chromatogram of both the solvents of *Q. infectoria* displayed the existence ten major peaks (Fig. 1, 2 and 3) and the component of the peaks were determined as follows gallic acid, tannic acid, protocatecunic acid, catechins, tannin, caffeic acid, chlorogenic acid, vanillic acid, coumaric acid, luteolin, quercetin. The concentrations of the bioactive compounds were determined

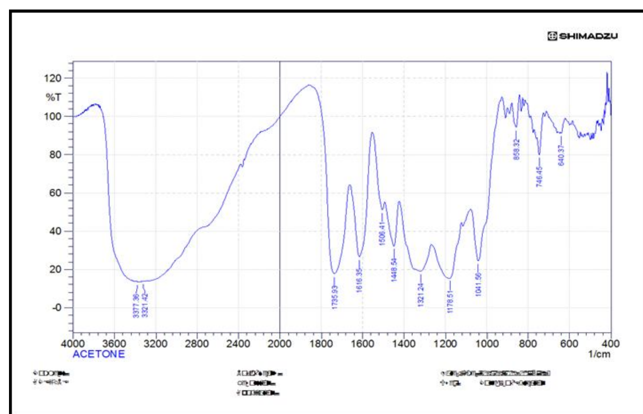

Fig. 5: FT-IR peak values of acetonic extract of *Quercus infectoria*.

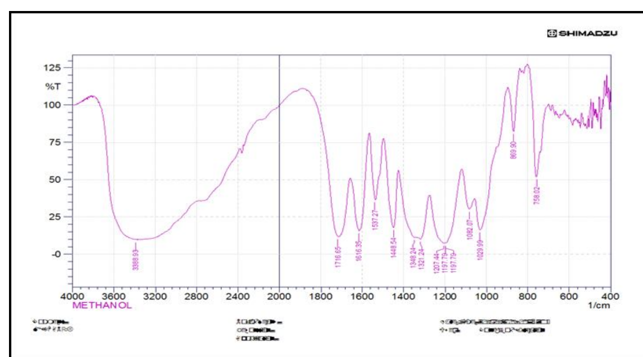

Fig. 6: FT-IR peak values of methanolic extract of *Quercus infectoria*.

by using peak area from the calibration curve (Fig. 4). As illustrated in table1, *Q.infectoria* contains high concentration of bioactive compounds by both types of solvent utilized in the method of extraction. These results indicate that methanol and acetone solvents for extraction of bioactive compounds from galls of *Q.infectoria* were the best two extraction solvents. This higher amount of these compounds could lead to pharmaceutical used such

Table 4: Antibacterial activity of extract against some pathogenic bacteria.

No.	Isolated pathogenic bacteria	Zone inhibition (mm)			
		Antibiotic control (Tetracycline) inhibition zone	Plant extract in micro.gm		
			20	30	40
1	*Staphylococcus aureus*	9	5	6	8
2	*Pseudomonus aeruginosa*	8	4	5.5	7
3	*E.coli*	9	9	10	11.5

as anticancer, anti-inflammatory and antioxidant activity as reported previously.

In the range of wave length 400 to 3600nm (Fig. 5 and 6), Evaluation of the *Quercus infectria* spectrum by FT-IR showed different peaks representing unlike functional groups illustrated in table 2 and 3. FT-IR spectrum suggested alcohol, phenols, carboxylic acid in fig. 5 and 6, alkenes, acetone and methanol QI extract aromatic compounds, nitro compounds, alkanes, esters and amines (Table 2 and 3). Antibacterial based on herbs have tremendous medicinal impacts because they can accomplish the goal with reduced symptoms. Further plant-extracted antimicrobial research is needed today. The FTIR of the *Q.infectoria* of this study was shown the presence of alkenes, aliphatic fluoro and nitro compounds (Tables 2 and 3). Methanol and acetone concentrate were found to be capable of preventing the spread of all oral illnesses in this research. This demonstrates that QI of gall concentration includes a wide range of antibacterial compounds, making it a potentially great source of antimicrobial substance. Moreover, both extracts have been an increased inhibitory impact on *Staphylococcus aureus, Pseudomonus aeruginosa* and *E.coli.*

Table 3: FT-IR peak values of methanolic extract of *Quercus infctoria*.

No.	Peak (Wave number cm^{-1})	Functional group assignment	Group frequency
1.	758	C-H bending	Alkene
2.	869	C-H bending	Alkenes
3.	1029	C -F stretching	Aliphatic fluoro compounds
4.	1082	C-O stretching	Carboxylic acid, ether, ester, alcohol
5.	1119	C-O stretching	Carboxylic acid, ether, ester, alcohol
6.	1321	$C-NO_2$	Nitro compounds
7.	1448	C-H	Alkane
8.	1537	N-H bending	Amine
9.	1616	N-H bending	Amine
10.	1716	C-H bending	Phenyl group substitution
11.	3155	O-H	Hydrogen bonded alcohols, phenols
12.	3280	O-H	Hydrogen bonded alcohols, phenols
13.	3338	O-H	Hydrogen bonded alcohols, phenols
14.	3340	O-H	Hydrogen bonded alcohols, phenols

The results (Table 4) explored the inhibition of the extract against three pathogenic microorganisms *Staphyloco- ccus aureus, Pseudomonus aeruginosa* and *E.coli*, isolated from the medical laboratory. While, more effect was on *E.coli, Staphylococcus aureus* and *Pseudomonus aeruginosa*, respectively. As the concentration increased, there was an increase in the inhibition area. The destruction of bacterial cells lead from the digestion of nucleic acids by fractions of the hydrogen bonds that link the nitrogen bases with each other, leading in DNA damage. Some researchers discovered

that some alkaloids work against microo-rganisms by influencing the cell wall.

Conclusion

Q. Infectoria of galls is a Kurdish native plant. IQ further confirmed that in folkloric medicine the plant extracts could be used for different circumstances. The HPLC and FT-IR analysis of methanol and acetone extract showed extremely complicated profile containing approximately ten compounds. This study proved that the extracts of the galls of *Q. infectoria* have high capability as antibacterial operator. This finding provides an insight to the use of these galls in traditional bacterial infection-related oral disease therapy. In addition, it can be used effectively in the clinical treatment of periodontal disease as a supplementary agent. Further investigations, however, are required to study, its other pharmacological and medicinal activities, attempts must be made to discover pharmaceutical products or even more advantageous biomedicines from biologically active crude extracts of this plant.

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