Separation and Identification of phenolic compounds from the flowers of Hibiscus sabdariffa L.and study its effect on two types of Bacteria

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Abstract
The current study focused on the phytochemical screening and antibacterial activity of two bacterial species *Acinetobacter baumannii* and *Streptococcus pneumoniae*, where we were able to isolate and identify some active compounds from the flowers of the Iraqi plant, Hibiscus sabdariffa L. This was achieved by preparing some plant extracts using the continuous extraction apparatus, Soxhlet, according to the successive solvent system which relies mainly on the difference in polarity, including petroleum ether (60-80) °C (HS1), chloroform (HS2), acetone(HS3), IMS(HS4), in addition to using hot water (HS5).Acid hydrolysis was performed on the raw extracts of acetone (HS3), IMS(HS4) and hot water (HS5) to obtain some free phenolic compounds, including (Chlorogenic acid,Caffeic acid,Rutin,Gallic acid, Quercetin, Ferulic acid) using high-performance liquid chromatographic (HPLC) technique. Ferulic acid appeared at the highest concentration in the IMS (HS4) extract compared to other extracts, while Rutin appeared at the lowest concentration in the acetone extract (HS3).*Hibiscus sabdariffa* Flowers were also tested for their effect on two types of bacteria, *Streptococcus pneumoniae* (G+) *and Acinetobacter baumannii* (G-), commonly found in human infections.Phenolic compounds were identified in the extracts, with the highest inhibition (43.65 mm) caused by the hot water extract (HS5) at 100% concentration, and the lowest inhibition (14.54 mm) caused by the IMS extract (HS4) at 25% concentration in *A. baumannii*.In *S. pneumoniae*, the highest inhibition (38.63 mm) was caused by the hot water extract (HS5) at 100% concentration and the lowest inhibition (10.36 mm) was caused by the acetone extract (HS3) at 25% concentration.
Key words: *Hibiscus sabdariffa* L., *Acinetobacter baumannii*, *streptococcus pneumoniae*, phenolic compounds, acid hydrolysis

Introduction

Medical plants have been used since ancient times. for their therapeutic properties. Recent research has shown that these plants contain bioactive that have the potential to interact with microorganisms and provide a Source of new antimicrobial agents (vaou et al., 2021).
Several medicinal plants have been found to exhibit antimicrobial activity against arange of microorganisms, for example, *Hibiscus sabdariffa* L. commonly known as the Roselle or the Guinea Sorrel, is a tropical plant species belonging to the family Malvaceae. It is native to west Africa but is now widely cultivated in several countries worldwide (El-Kinany et al, 2020)
The plant reaches between 1.5 and 3.5 m (Alara and Abdurahman, 2019). Its leaves are Simple, hanging with a toothed edge, lobed palmarily, with 3-5 alternating lobes placed on the stem, green or red with aneck length (2-10) cm, in the leaf there is a nectar gland at the base of its main vein (Al-Atrakji et al., 2019).
The flowers are Single, whole, axillary large and with five petals. (Singh et al., 2021). Its stems are rigid, cylindrical, un branched or Sub basal usually red and green and sometimes with red dots (Izqierdo Vega et al., 2020).
H. Sabdariffa L. is an abundant and interesting Source of bioactive molecules (compounds produced by plants that have pharmacological effects). (Ojulari et al., 2019) Such as polyphenols, flavonoids, phytosterols, saponins, lignins, essential oils, flavanols in Simple or polymeric form (Piovesana et al., 2019)
The plant shows antibacterial, anti-parasitic, anti-inflammatory, anti-oxidant, anti-cancer, anti-spasmodic, anticonvulsant as well as the calyx extract is used as an effective treatment for patients suffering from Kidney stones. It stimulates the intestines, improves digestion in addition to its pressure _ preserving ability, it is also used in folk medicine as a diuretic, antipyretic and used to treat cardiovascular diseases and atherosclerosis (Nguyen et al., 2022; Singh et al., 2021).
The discovery of new antimicrobial agents is becoming increasingly urgent as the global burden of antibiotic-resistant infections continues to rise. Medicinal plants represent a promising source of new antimicrobial agents, as they have been used for centuries to treat a variety of infections. Recent research has identified several promising bioactive compounds from medicinal plants that have potent antimicrobial activity against a range of microorganisms. These compounds may serve as lead molecules for the development of new drugs that can combat antibiotic-resistant infections. (Serwecińska, 2020).
Acinetobacter baumannii is a gram-negative bacterium aerobic ،non-motile ,swollen commonly found in hospital environments, particularly in intensive care units. It has become a major

doi.org/10.31838/hiv23.03.202 Received: 17.11.22, Revised: 26.12.22, Accepted: 24.01.23.

healthcare- associated pathogen due to its ability to develop resistance to multiple classes of antibiotics, (Howard et al.,2012) These bacteria is one of the most important pathogens of opportunistic diseases, and its infections are dangerous, including putrefaction Septicemia and Bacteremia cause meningitis and urinary tract infection and Pneumonia, wound and burn injuries, inflammation of the cornea of the eye, brain and bone inflammation.(Almasaudi, 2018)

A. baumannii has been shown to produce several virulence factors that contribute to its pathogenicity, including outer membrane proteins, lipopolysaccharides, and capsule polysaccharides. These factors are involved in the bacterium's ability to adhere to and invade host cells, evade the host immune response, and form biofilms2012 (Cho & Blaser , 2012)

Streptococcus pneumoniae, commonly known as pneumococcus, is a Gram-positive bacterium facultative anaerobic, non-motile, It is considered one of the fastidious bacteria, because it requires growth promoters, as it does not grow on normal media, but rather it needs media rich in traces of blood and cooked blood cultures, and it needs CO2 gas by (7-5) %, (McDevitt, et al.,2020) that is a significant cause of morbidity and mortality worldwide. It is responsible for a wide range of diseases, including pneumonia, meningitis, and sepsis, particularly in young children, the elderly, and immunocompromised individuals (Brooks et al., 2018) Recent studies have shed new light on the virulence mechanisms employed by *S. pneumoniae*, providing insights into the host-pathogen interactions that underlie disease development. For example, the pneumococcal capsule, a thick layer of polysaccharides that surrounds the bacterium, has long been known to contribute to bacterial virulence by preventing phagocytosis and complement-mediated killing. However, recent studies have identified additional virulence factors, including pneumolysin, Hemolysin , Neuramindase, Autolysin, a pore-forming toxin that damages host cells, and the choline-binding proteins, which promote bacterial colonization and evasion of the immune system.(Liu et al., 2022)

Taxonomical classification	
Biological name	Hibiscus sabdariffa L.
Kingdom	Planta
Sub_ Kingdom	Tracheobionta
Division	Mangolipophy
Sub_ Division	Angiospermae
Class	Mangoliopsida
Order	Malvales
Family	Malvaceae
Genus	Hibiscus
Species	*Hibiscus sabdariffa L.*
(ITIS , 2010)	

Domain	Bacteria
Phylum	Bacillota
Class	Bacilli
Order	Lactobacillales
Family	Streptococcaceae
Genus	Streptococcus
Species	Pneumoniae
(Bergey, 2012)	

Domain: Bacteria	Domain: Bacteria
Phylum	Proteobacteria
Class	Gamaproteobacteria
Order	Pseudomonodales
Family	Moraxellaceae
Genus	Acinetobacter
Species	Baumannii
(Bouvet & Grimont, 1986)	

Materials and Methods

Collection of the flowers

The flowers of the Iraqi Plant *H. Sabdariffa* L. were collected from markets in Mosul in the year (2021-2022), and were classified by Dr. Ameer Mohsen Mahmoud, professor of taxonomy at the university of Mosul / college of Education for Pure Science

After that, the flowers were cleaned from the dust and grinded, then put in a paper batch. and kept in a place away from moisture and Sun rays until using them.

Preparation of some plant extracts by using Continuous (soxhlet) apparatus.

The flowers of *H.sabdariffa* L. were crushed by an electric mill, where 25 g of it, the well- ground powder was placed in the soxhlet batch system and 200 ml of petroleum ether (60-80) °c was added to the flowers extracted oil. The extraction process continued at a rate (6-7) hours per day until the solvent in the device became colorless.

In the end, the extract was concentrated by using a Rotary Vacuum Evaporator (RVE) (Harborne, 1984).

Four solvents were used in the soxhlet apparatus by sequence solvent system concept petroleum ether (60-80) °C (HS1), chloroform (HS2), Acetone (HS3), IMS (HS4), hot aqueous extract (HS5) was carried out using Grand method. (Le Grand et al., 1998).

Acid hydrolysis process

10 ml of crude acetone extract (HS3) and IMS extract (HS4) and hot water extract (HS5) were taken each separately, and (25) ml of (IN) Hcl was added to it, after which the reflux was done at 100°C for 60 minutes, and the Solution was left to cool down after that the solution was placed in the separating funnel and 50 ml of ethyl acetate was added to it twice with continuous shaking. Then two layers were formed the upper layer (organic layer) which is the layer of ethyl acetate and bottom layer, the upper layer was taken and (3) g of $MgSo_4$ was added to it, the samples were Kept in tightly covered glass bottles and placed in the refrigerator until they were identified by the HPLC device (Al Mashhadani, 2020; Al Zaidi & Khorsheed, 2021).

The phenolic compounds detected depending on the area of the compound and as the percentage ratio of the separated compound or they were converted to concentrations (mg. g^{-1}) according to the previously approved equation (Behbahani et al., 2011)

Identification of phenolic compounds using HPLC-UV device

Phenolic compounds were identified in Baghdad in the

laboratories of the Ministry of Science and technology/Dept. of Environment and water resources after conducting the acid hydrolysis process.

According to the method presented before (Mradu et al., 2012) by using high-performance liquid chromatography device HPLC type sykam of German origin with a flow rate of 1.3 (ml min. -1), The mobile phase is (A) which include (Methanol: D.W: formic acid, (70:25:5) with the column (18-0DS) has dimensions (25 cm * 4.6mm) and the responses were detected at the UV - 360 nm. wave length.

Sensivity test method (diffusion by pits)

The antimicrobial susceptibility test was done using well diffusion method and concentrations (100, 75, 50,25) of plant extracts were prepared after the acid hydrolysis process.

Muller _Hinton agar was prepared by adding 38g of Muller -Hinton agar to 1 Liter of distilled water then mixed well with heating using hot plate magnetic stirrer until boiling, the pH was adjusted at 7.4, After that, the agar was autoclaved at 115°C pressure 15 pounds/inch for 15-20 min. using protable autoclave. The agar was cooled and poured in to sterile petri dish and left to solidify these petri dishes are used for *Acinetobacter baumannii*. As for *Streptococcus pneumoniae*, after Muller- Hinton agar was cooled to 45-50 °C, human blood free from contamination was added to it at a rate of 5-10%. per 100 ml of the medium and mixed well then poured in to sterile petri dish and left to solidify (Traub & Leonhard, 1996).

After that, 6 mm diameter wells were made in the agar plates Fresh cultures were prepared of *Acinetobacter baumannii* and *Streptococcus pneumoniae* and adjusted to 0.5 McFarland standard. The agar plates were inoculated with specific bacteria using sterile cotton swab immersed in the bacterial suspension and spread on the surface of Muller-Hinton agar for *A. baumannii* and Muller-Hinton agar added blood for*S. pneumoniae* and left for 15 min., after that 0.1 ml of the extract with different concentrations was loaded in the corresponding wells, and standard antibiotic disc (ciprofloxacin) was used as control positive while 0.1 ml of DMSO was used as control negative incubated for 37°C for 24 h for *A. baumannii*, As for the *S. pneumoniae* petri dishes were incubated at 37°C with the provision of CO2 gas. The inhibition Zone was measured using digital caliper (perez et al., 1990).

Result and Discussions

Identification of number of phenolic compounds of Hibiscus sabdariffa L. flowers by using HPLC- UV device

The chart of analysis obtained shows that the retention time of each sample was obtained and compared with standard, the retention time of Chlorogenic acid (2.80 min.), Caffeic acid (4.28 min), Rutin (5.89 min), Gallic acid (7.92 min.), Quercetin (9.96 min) and ferulic acid (11.92 min.) Table (1), fig (1,2,3,4,5,6).

Table (1) Indicated the standard retention times and the concentration of some phenolic compounds by using HPLC technique of *H. Sabdariffa* L. flowers.

No.	Standard Phenolic Compounds	Standard of Retention Time (min.)	Acetone extractHS3		IMS extractHS4		Hot aqueous extract HS5	
			The Concentration mg/g	Retention Time	The Concentration mg/g	Retention Time	The Concentration mg/g	Retention Time
1	Chlorogenic acid	2.80	0.009785	2.78	0.0105949	2.72	0.01021168	2.74
2	Caffeic acid	4.28	0.0213056	4.26	0.0227443	4.24	0.0219689	4.26
3	Rutin	5.89	0.0060416	5.88	0.0068040	5.88	0.0065379	5.84
4	Gallic acid	7.92	0.0136663	7.90	0.01692592	7.99	0.0141728	7.99
5	Quercetin	9.96	0.026654	9.90	0.0300968	9.92	0.0272735	9.98
6	Ferulic acid	11.92	0.0332024	11.90	0.03681176	11.93	0.03593208	11.98

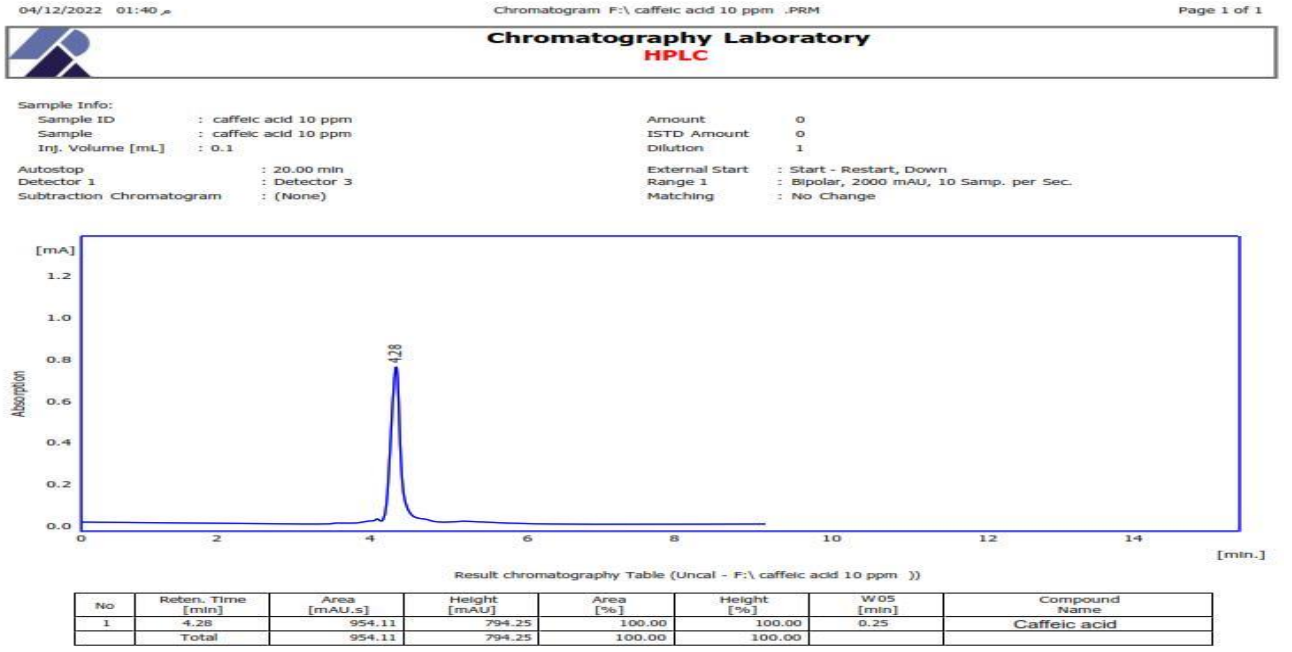


Figure (1) The standerd curve of chlorogenic acid

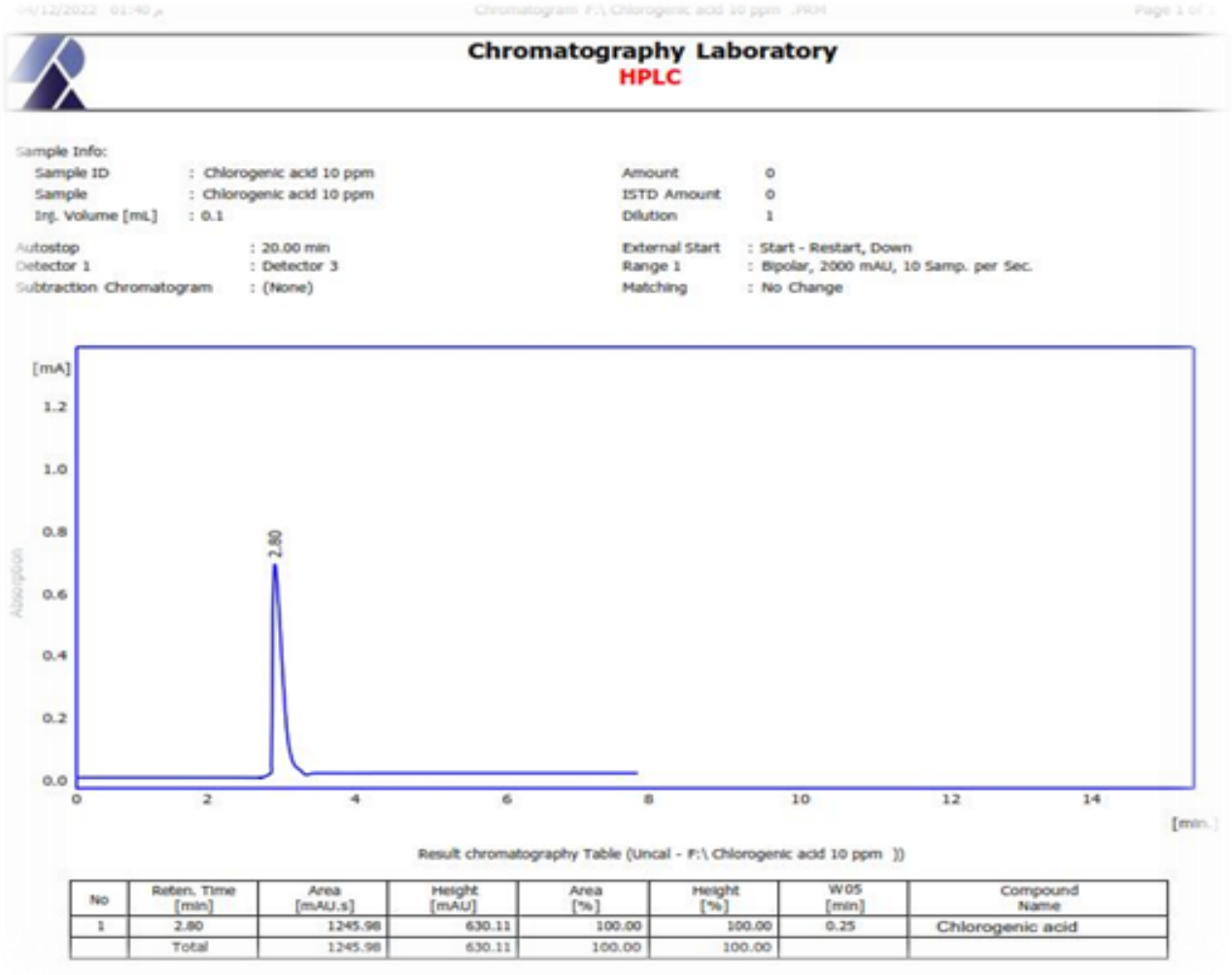


Figure (2) The standerd curve of caffeic acid

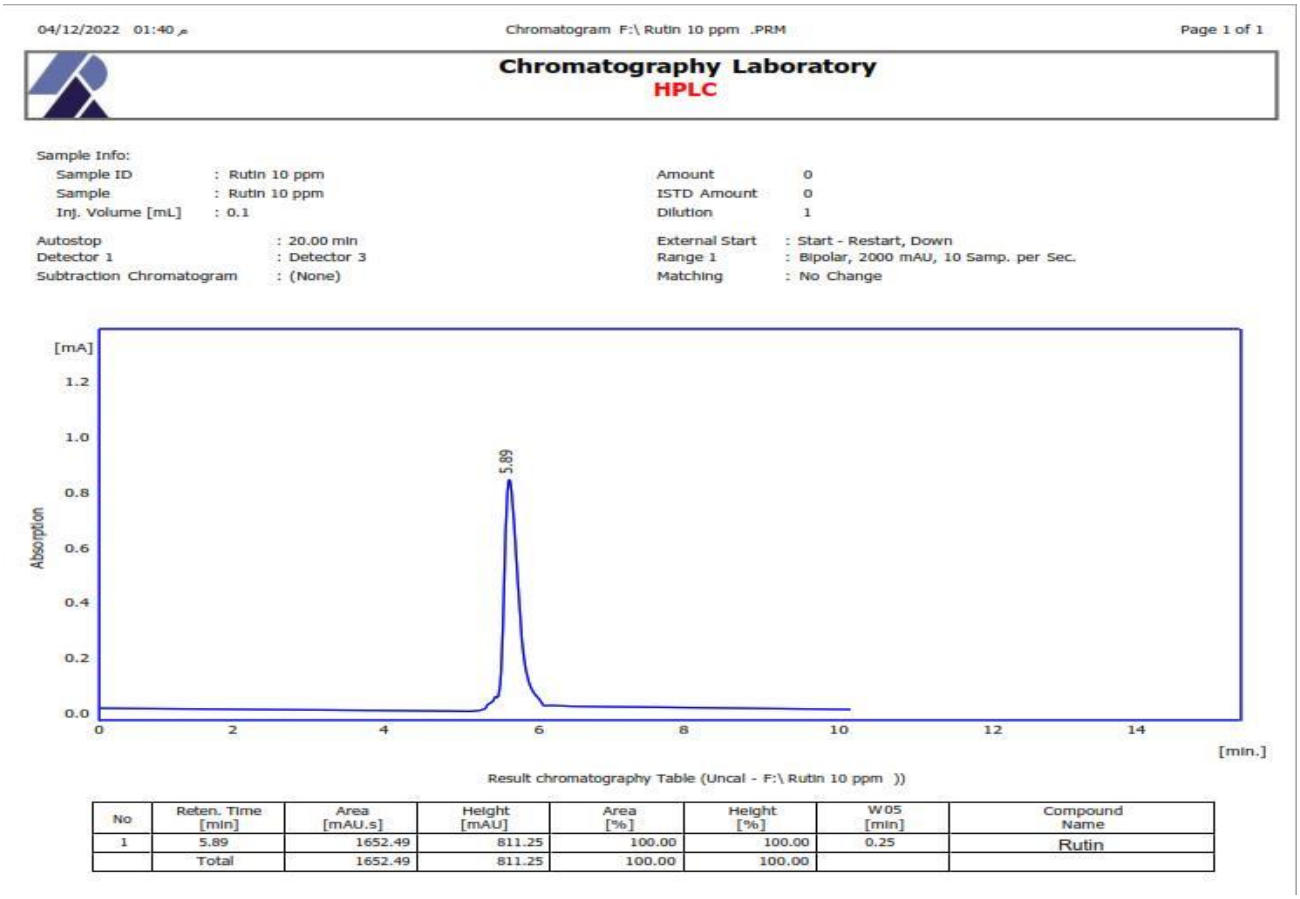


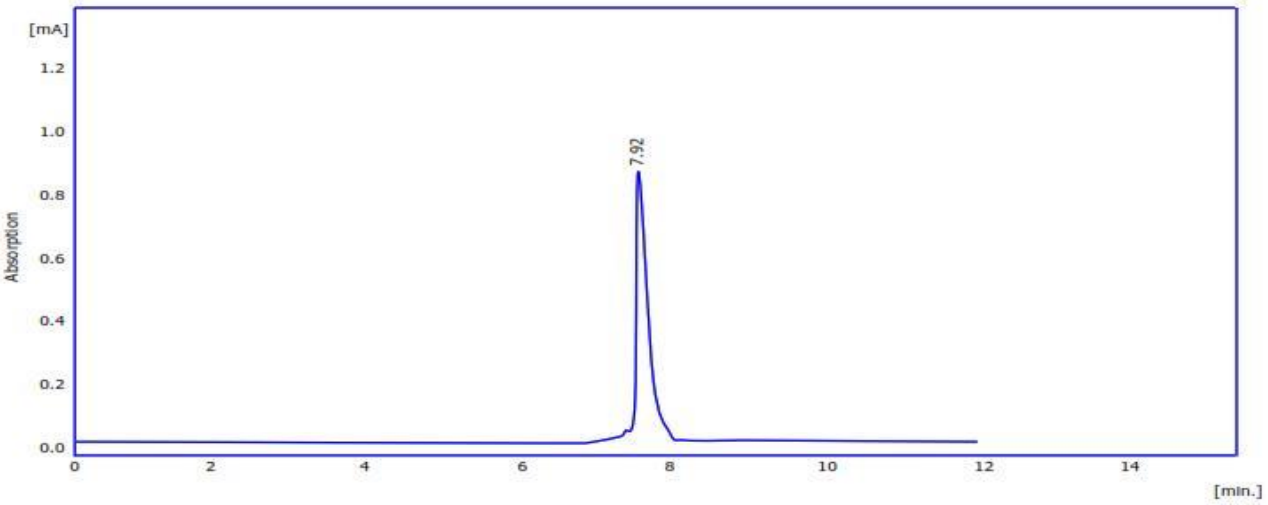
Figure (3) The standerd curve of rutin

04/12/2022 01:40 م Chromatogram F:\ gallic acid 10 ppm .PRM Page 1 of 1

Chromatography Laboratory
HPLC

Sample Info:

Sample ID	: Gallic acid 10 ppm	Amount	0
Sample	: Gallic acid 10 ppm	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ gallic acid 10 ppm))

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	7.92	1874.08	962.18	100.00	100.00	0.25	Gallic acid
	Total	1874.08	962.18	100.00	100.00		

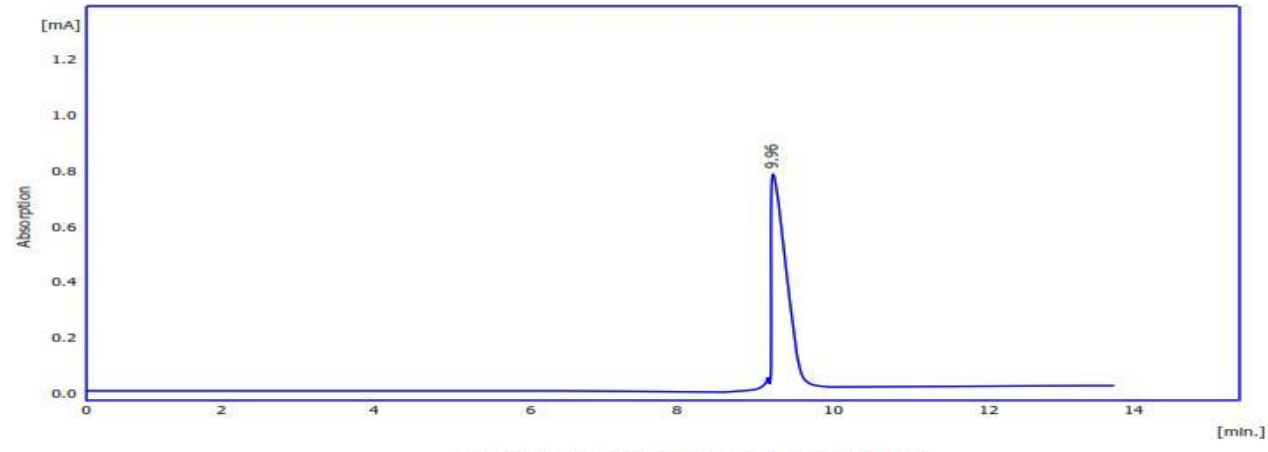
Figure (4) The standerd curve of gallic acid

01/12/2022 01:40 م Chromatogram F:\ qurcetine (10 PPM) .PRM Page 1 of 1

Chromatography Laboratory
HPLC

Sample Info:

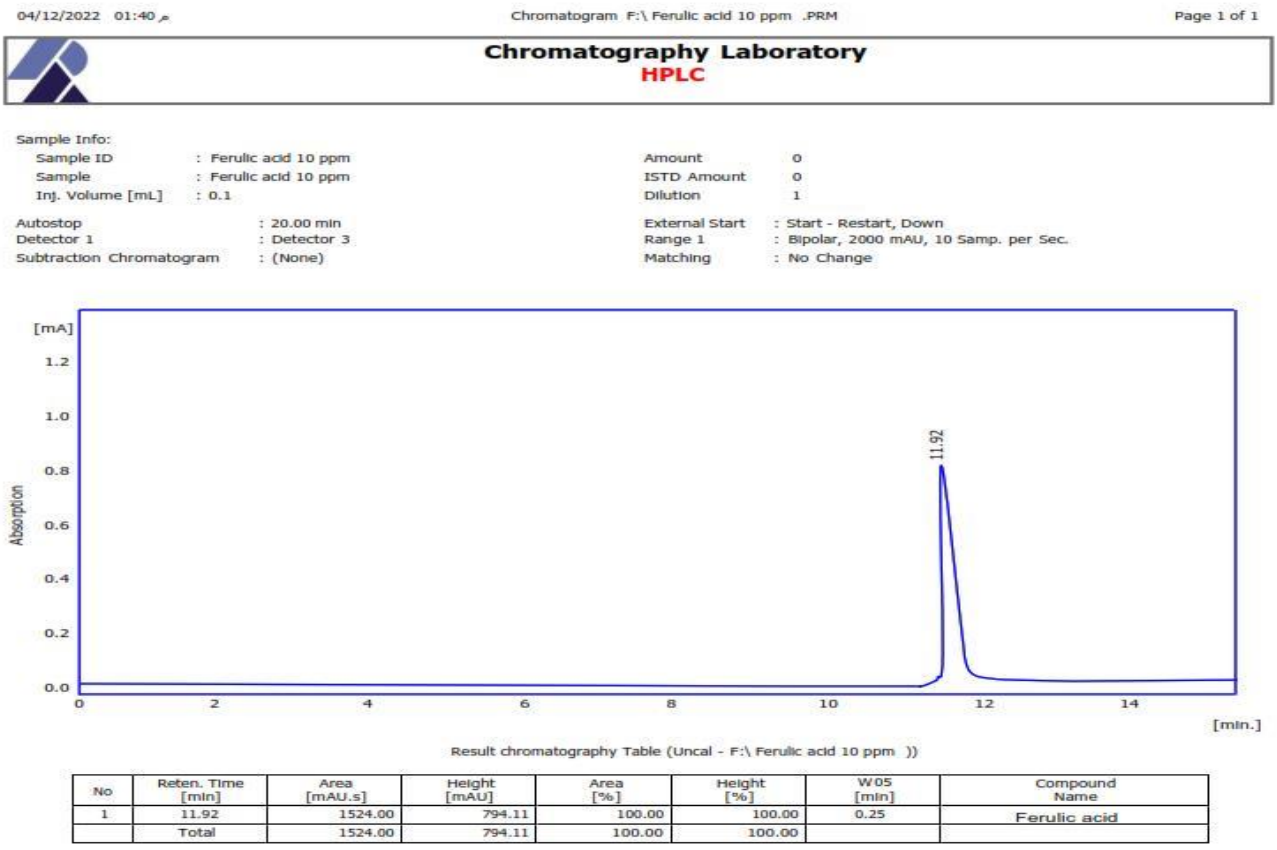
Sample ID	: Qurcetine (10 PPM)	Amount	0
Sample	: Qurcetine (10 PPM)	ISTD Amount	0
Inj. Volume [mL]	: 0.1	Dilution	1
Autostop	: 20.00 min	External Start	: Start - Restart, Down
Detector 1	: Detector 3	Range 1	: Bipolar, 2000 mAU, 10 Samp. per Sec.
Subtraction Chromatogram	: (None)	Matching	: No Change



Result chromatography Table (Uncal - F:\ qurcetine (10 PPM))

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	9.96	1574.15	799.58	100.00	100.00	0.25	Quercetin
	Total	1574.15	799.58	100.00	100.00		

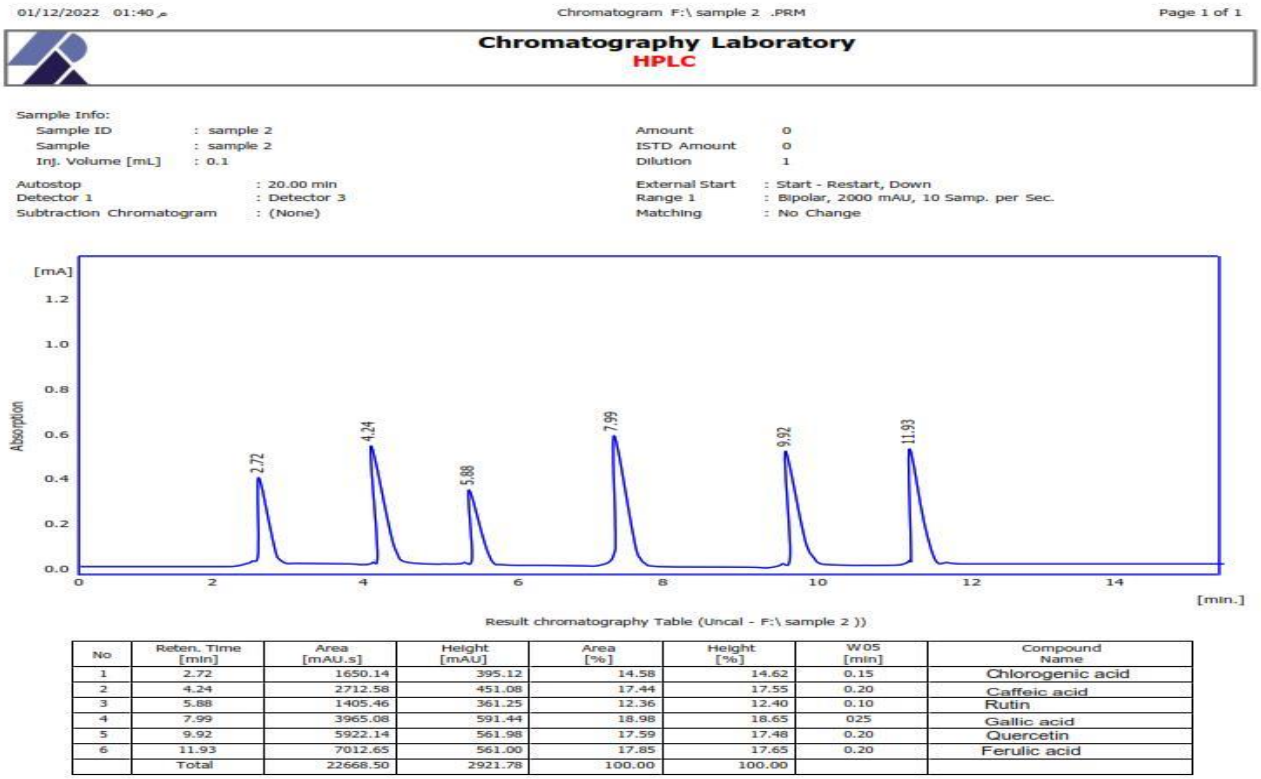
Figure (5) The standerd curve of quercetin



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	11.92	1524.00	794.11	100.00	100.00	0.25	Ferulic acid
	Total	1524.00	794.11	100.00	100.00		

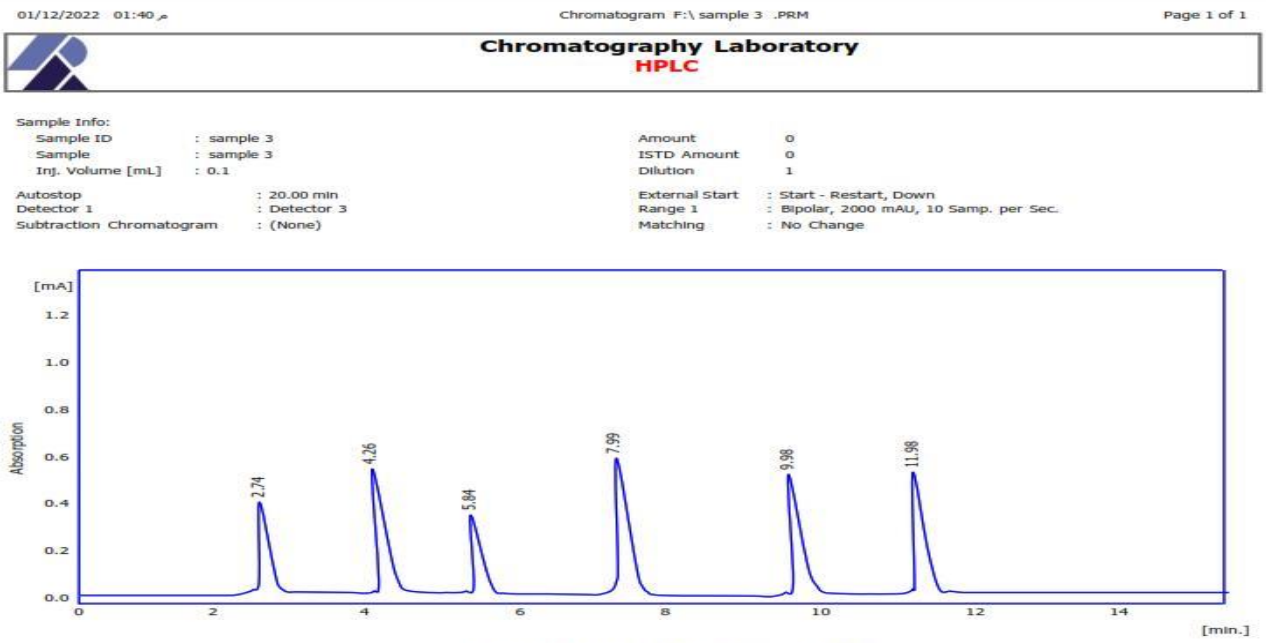
Figure (6) The standerd curve of ferulic acid

This indicates the presence of the phenolic compounds in the flowers of *H. Sabdariffa* L. Chlorogenic acid was showed in three extracts (HS1. HS2, HS3) after acid hydrolysis process. The concentrations of chlorogenic acid were (0.009785 ,0.0105949, 0.01021168) (mg. g-¹), caffeic acid were (0.0213056. 0.0227443, 0.0219689) (mg. g-¹), rutin were (0.0060416, 0.0068040, 0.0065379) (mg. g-¹), gallic acid were (0.0136663, 0.01692592, 0.0141728) (mg. g-¹), quercetin was (0.026654, 0.0300968, 0.0272735) (mg. g-¹) and ferulic acid (0.0332024, 0.03681176, 0.03593208) (mg. g-¹). Table (1) and fig. (6,7,8)



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.72	1650.14	395.12	14.58	14.62	0.15	Chlorogenic acid
2	4.24	2712.58	451.08	17.44	17.55	0.20	Caffeic acid
3	5.88	1405.46	361.25	12.36	12.40	0.10	Rutin
4	7.99	3965.08	591.44	18.98	18.65	025	Gallic acid
5	9.92	5922.14	561.98	17.59	17.48	0.20	Quercetin
6	11.93	7012.65	561.00	17.85	17.65	0.20	Ferulic acid
	Total	22668.50	2921.78	100.00	100.00		

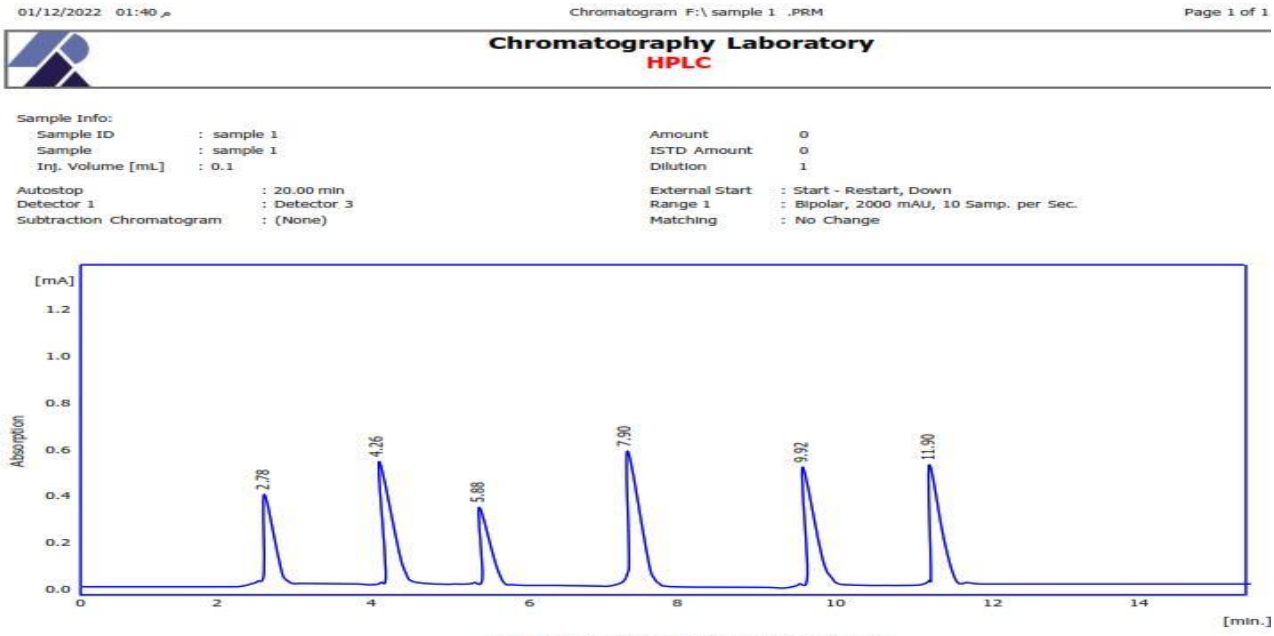
Figure (7): The phenolic compounds from the acid hydrolysis acetone extract (HS3)



Result chromatography Table (Uncal - F:\ sample 3))

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.74	1590.45	394.35	14.58	14.62	0.15	Chlorogenic acid
2	4.26	2620.11	450.48	17.44	17.55	0.20	Caffeic acid
3	5.84	1350.49	360.50	12.36	12.40	0.10	Rutin
4	7.99	3320.15	590.64	18.98	18.65	025	Gallic acid
5	9.98	5366.58	560.30	17.59	17.48	0.20	Quercetin
6	11.98	6845.07	560.48	17.85	17.65	0.20	Ferulic acid
	Total	21092.59	2916.48	100.00	100.00		

Figure (8): The phenolic compounds from the acid hydrolysis IMS extract (HS3)



Result chromatography Table (Uncal - F:\ sample 1))

No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.78	1524.08	394.25	14.58	14.62	0.15	Chlorogenic acid
2	4.26	2541.00	450.11	17.44	17.55	0.20	Caffeic acid
3	5.88	1247.98	360.11	12.36	12.40	0.10	Rutin
4	7.90	3201.49	590.41	18.98	18.65	025	Gallic acid
5	9.90	5244.87	560.25	17.59	17.48	0.20	Quercetin
6	11.90	6325.08	560.14	17.85	17.65	0.20	Ferulic acid
	Total	20084.50	2915.45	100.00	100.00		

Figure (9): The phenolic compounds from the acid hydrolysis hot aqueous extract (HS3)

Effect the active compounds for the flowers of Hibiscus sabdariffa L. in Acinetobacter baumannii and Streptococcus pneumoniae by well diffusion method

The inhibitory activity was tested for some the plant extracts (Acetone. IMS, Hot aqueous) after acid hydrolysis and by four concentrations (25, 50 75, 100) % for two types of the using bacteria in the study to Know the inhibitory effect of each type from the bacteria, and each concentration. from the different extracts, table (2) Picture (1) The results was showed that the hot aqueous extract (HS5) showed high effect for *A. baumannii* that exceeds the effect of

antibiotics ciprofloxacin the inhibitory diameter was reached at the concentration 100%. (43.65) mm and (42.04) mm, in concentration 75% while in concentration 50% (36.02) mm and (27.64) mm in concentration 25% compared to (21.63) mm for the antibiotic ciprofloxacin.Table(2), fig (10).

IMS extract (HS4) showed a high inhibition effect for *A. baumannii* with inhibitory diameter was reached at concentrations 100% and 75% (28.55) mm، (23.88) mm respectively compared to (20.08) mm for the antibiotic ciprofloxacin Picture (2) while the concentrations of 50% and 25% showed less effect than the antibiotic with inhibitory diameter (20.13) mm and (14.54) mm respectively.

As for the acetone (HS3) showed a high inhibition effect for *A. baumannii* at amount (29.05) mm in concentration 100% compared to (21.42) mm for the antibiotic ciprofloxacin and a close effect with the antibiotic at a concentration of 75% (20.56) mm compared to (21.42) mm for the used antibiotic, while concentrations of 50% and 25% showed a less effect (16.36) mm (15.04) mm, respectively, as in the picture (3)

And the results of the current study Abdallah (2016) who used ethanol extract and showed an inhibitory effect on *A. baumannii* bacteria with an inhibition diameter of (13.6) mm using the disc diffusion method

Table (2) and fig. (10) showed that the hot aqueous extract (HS5) when used in concentration (25, 50, 75, 100) % appeared a high inhibition for *S. pneumoniae* all of them were superior to the control treatment (ciprofloxacin) as the inhibitory diameter reached at (38.63) mm, (34,97) mm, (30.41) mm and (27.25) mm, respectively, compared to (24.94) mm for ciprofloxacin, Picture (4)

And that the IMS extract (HS4) showed a high inhibitory effect at a concentration of 100% only with an inhibitory diameter of (30.43) mm compared to (24.06) mm for Ciprofloxacin and at a concentration of 75%, 50% and 25% gave less inhibition than the inhibition of antibiotics used with a diameter of inhibition of (22.85) mm, (18.63) mm, and (11.21) mm, respectively, as in picture (5)

while the acetone extract with all its concentrations (25, 50, 75, 100) % gave moderate and less inhibition than the antibiotic Ciprofloxacin with an inhibition diameter of (21.00) mm, (17.14) mm, (14.59) mm, and (10.36) mm. respectively, compared to (23.97) mm for the antibiotic, as in the picture (6).

The results of the current study are consistent with Zimmerman and Ibrahim (2022) in the ability of phenols On the inhibition of *S.pneumoniae* bacteria.

Table (2): The Effect of concentration of some phenolic extracts from flower of Iraqi Hibiscus sabdariffa L. on two type of Bacteria (A. baumannii, S. pneumonia)

Type of Extract	Concentration	*A. baumannii*	*S. pneumoniae*
Acetone extract HS3	100%	29.0567 g.66606	21.0033 mno.85909
	75%	20.5633 no.79002	17.1400 q.36042
	50%	16.3633 q.60863	14.5900 r.43209
	25%	15.0400 r.76896	10.3600 s.31749
	HS3 cip	21.4200 mn.75901	23.9700 jk.22716
IMS extractHS4	100%	28.5567 gh.80164	30.4367 f.60871
	75%	23.8800 k1.38156	22.8500 l.65092
	50%	20.1333 o.33471	18.6367 p.43097
	25%	14.5433 r.54994	11.2133 s.19502
	HS4 cip	20.0800 o.22605	24.0667 jk.24906
Hot aqueous HS5	100%	43.6533 a.60517	38.6333 c.38940
	75%	42.0467 b.79412	34.9767 e.93575
	50%	36.0200 d.11790	30.4167 f.30238
	25%	27.6433 hi.24786	27.2533 i.49642
	HS5 cip	21.6333 m.66229	24.9467 j.18556
	DMSO	.0000 t.00000	.0000 t.00000

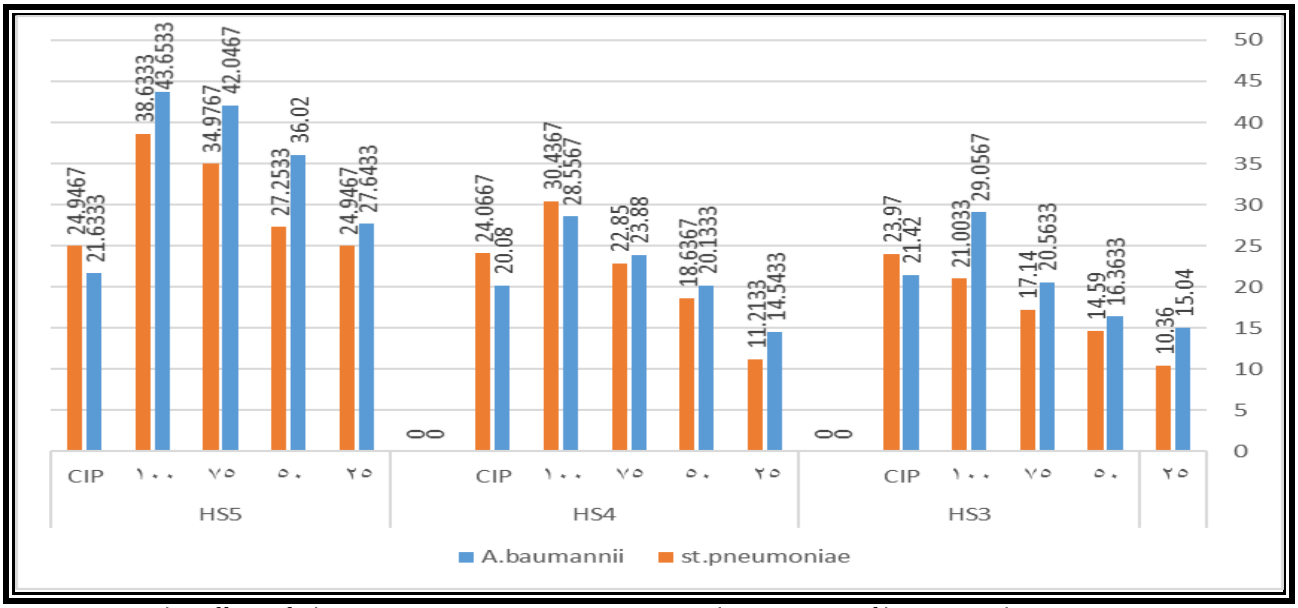
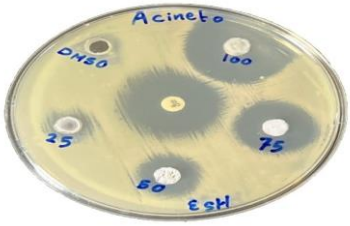
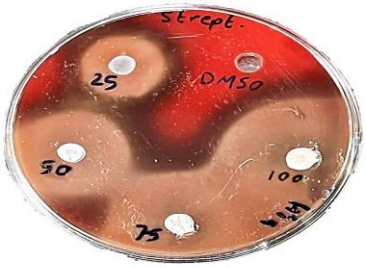


Figure (10): The effect of plant (Acetone,IMS,Hot aqueous) on the two type of bacteria A.baumannii ,S.pneumoniae



Pic. (3): The effect of Hot aqueous extract on A. baumannii (G+)

Pic. (5): The effect of IMS extract on S. pneumoniae (G-)



Pic. (6): The effect of Acetone extract on S. pneumonia (G-)

Conclusion

The phenolic compounds were showed in different extracts from the flower of Hibiscus sabdariffa L. which is growing in Iraq and investigated by using chromatographic analysis HPLC-technique

The results showed that the phenolic compounds had a clear effect on inhibition in two type of bacteria are *Acinetobacter baumannii* and *Streptococcus pneumoniae*

The hot water extract (HS5) at concentrations (25, 50, 75, and 100%) showed high significant inhibition against *S.pneumoniae* and *A.baumannii* because it is known that the Gujarat plant contains many phenolic compounds, flavonoids and tannins, which act as active ingredients against bacteria, including ferulic acid, which appeared in high concentrations in the plant extracts of H. Sabdariffa L., and it is a powerful antioxidant and has antioxidant properties, as well as Quercetin, which is known for its activity against bacteria

Acknowledgments

Many thanks go to the staff of Department of Biology, College of Education for Girls. University of Mosul for the logistic help it has provided.

References

Abdallah, E. M. (2016). Antibacterial activity of *Hibiscus sabdariffa* L. calyces against hospital isolates of multidrug resistant *Acinetobacter baumannii*. Journal of Acute Disease, 5(6), 512-516.

Al- Atrakji, A. O., Al -Daody, A. C. & Al-Mawsili,M. A. (2019), Medicinal and Aromatic plants.Dar Ibn Al-Atheer for printing and publishing, university of Mosul, Iraq , 301-302.

Alara, O. R., & Abdurahman, N. H. (2019). Microwave-assisted extraction of phenolics from *Hibiscus sabdariffa* calyces: Kinetic modelling and process intensification. Industrial Crops and Products, 137, 528-535.

Almasaudi, S. B. (2018). Acinetobacter spp. as nosocomial pathogens: Epidemiology and resistance features. Saudi journal of biological sciences, 25(3), 586–596.

Al-Mashhadani, M. M. B. (2020). "Fiber dimensions effect in paper manufacturing and estimating some natural products of Ailanthus swingle tress grown in Mosul city, M.Sc. thesis, College of Agriculture and Forestry, University of Mosul.

Al-zaidi, Y. M. and Khorsheed, A. C. (2021). Separation and identification of many volatile oil compounds and phenolic compounds from the seeds of *Ammi visnaga* (L .) growing in Iraq , Journal of Kerbala for agricultural Sciences ,8 (2), 1–11.

Behbahani, M., Shanehsazzadeh, M., & Hessami, M. J. (2011). Optimization of callus and cell suspension cultures of Barringtonia racemosa (Lecythidaceae family) for lycopene production. Scientia Agricola, 68, 69-76.

Bergey, D. H. Good fellow, M., Whitman, W.B., & Parte, A.C. (2012). The Actinobacteria. Bergey's manual of systematic bacteriology.

Bouvet, P.J., & Grimont, P.A. (1986). Taxonomy of the genus Acinetobacter with the recognition of *Acinetobacter baumannii* sp. nov., *Acinetobacter haemolyticus* sp. nov., *Acinetobacter johnsonii* sp. nov., and *Acinetobacter junii* sp. nov. and emended descriptions of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii*. International Journal of Systematic and Evolutionary Microbiology, 36(2), 228-240.

Brooks, L. R., & Mias, G. I. (2018). *Streptococcus pneumoniae*'s virulence and host immunity: aging, diagnostics, and prevention. Frontiers in immunology, 9, 1366.

Cho, I., & Blaser, M. J. (2012). The human microbiome: at the interface of health and disease. Nature reviews Genetics, 13(4), 260–270.

El-Kinany, R. G., Salama, Y. E., Rozan, M. A., Bayom, H. M., & Nassar, A. M. (2020). Impacts of humic acid, indole butyric acid (IBA) and arbuscular mycorrhizal

fungi (*Glomus mosseae*) as growth promoters on yield and phytochemical characteristics of *Hibiscus sabdariffa* (Roselle). Alexandria Science Exchange Journal, 41(JANUARY-MARCH), 29-41.
Harborne, J. B. (1984). Phytochemical Methods, 2nd ed; by Chapman and Hall. London and New York 1984, 37(3), 19-25.
Howard, A., O'Donoghue, M., Feeney, A., & Sleator, R. D. (2012). *Acinetobacter baumannii*: an emerging opportunistic pathogen. Virulence, 3(3), 243–250.
ITIS(2010).Integrated Taxonomic Information System on_line database Iwashina,T.(2015). Contribution to flower Colors of Flavonoids Including Anthocyanins:A Review . Natural Product Communications,10(3) : 529_544.
Izquierdo-Vega, J. A., Arteaga-Badillo, D. A., Sánchez-Gutiérrez, M., Morales-González, J. A., Vargas-Mendoza, N., Gómez-Aldapa, C. A., ... & Madrigal-Santillán, E. (2020). Organic acids from Roselle (*Hibiscus sabdariffa* L.)—A brief review of its pharmacological effects. Biomedicines, 8(5), 100.
Le Grand, A., Wondergem, P. A., Verpoorte, R., & Pousset, J. L. (1988). Anti-infectious phytotherapies of the tree-savannah of Senegal (West-Africa) II. Antimicrobial activity of 33 species. Journal of ethnopharmacology, 22(1), 25-31.
Liu, B., Zheng, D., Zhou, S., Chen, L., & Yang, J. (2022). VFDB 2022: a general classification scheme for bacterial virulence factors. Nucleic acids research, 50(D1), D912-D917.
McDevitt, E., Khan, F., Scasny, A., Thompson, C. D., Eichenbaum, Z., McDaniel, L. S., & Vidal, J. E. (2020). Hydrogen Peroxide Production by *Streptococcus pneumoniae* Results in Alpha-hemolysis by Oxidation of Oxy-hemoglobin to Met-hemoglobin. Msphere, 5(6), e01117-20.
Mradu, G., Saumyakanti, S., Sohini, M. and Arup, M. (2012). "HPLC profile of standard phenolic compounds present in medical plants. International Journal of Pharmacognosy and phytochemical research, 4 (3),162-167
Nguyen, N. N. (2022). Microencapsulation of roselle (*Hibiscus sabdariffa* L.) anthocyanins: Effects of different carriers on selected physicochemical properties and antioxidant activities of spray-dried and freeze-dried powder. International Journal of Food Properties, 25(1), 359-374.
Ojulari, O.V., Lee, S. G., & Nam, J. O. (2019). Beneficial effects of natural bioactive compounds from *Hibiscus Sabdariffa* Lon obesity Molecules, 24(1),210.
Perez, C., pauli, M. and Bazerque, P. (1990). An antibiotic assay by agar well diffusion methods. Acto biologiae et medicinae experimentalis, 15: 113-115.
Piovesana, A., Rodrigues, E., & Noreña, C. P. Z. (2019) Compart analysis of carotenoids and phenolic compounds and antioxidant activity from hibiscus calyces (*Hibiscus sabdariffa* L.) by. HPLC - DAD MS/MS. Phytochemical Analysis, 30 (2), 208-217.
Serwecińska, L. (2020). Antimicrobials and antibiotic-resistant bacteria: a risk to the environment and to public health. Water, 12(12), 3313.
Singh, D., Batra, K., Sharma, C., Kaur, N., Kaur, G., & Kapoor, M. (2021). The traditional uses, phytochemistry and pharmacology of Genus Hibiscus: a review. European Journal of Medicinal Plants, 32(4), 1-37.
Traub, W. H., & Leonhard, B. (1996). Antibiotic susceptibility of clinical isolates of *Streptococcus pneumoniae*. Chemotherapy, 42(4), 240-247.
Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., & Bezirtzoglou, E. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. Microorganisms, 9(10), 2041.
Zimmerman, T., & Ibrahim, S. A. (2022). Quercetin Is a Novel Inhibitor of the Choline Kinase of *Streptococcus pneumoniae*. Antibiotics, 11(9), 1272.