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ANTIBACTERIAL ACTIVITY FOR TOTAL PHENOLS AND TOTAL ALKALOIDS EXTRACTED FROM *NARCISSUS TAZETTA* L. BULBS

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Abstract

Narcissus tazetta plant from Amaryllidaceae family is known to be rich in bioactive metabolites such as Alkaloids, phenolics and flavonoid which detected in almost species of this family. *N. tazetta* cultivated in Iraq, had not yet been studied for its active components antimicrobial study; thus the current study employed the antibacterial activity for total phenolic compounds and total alkaloids extracted from this plant. The plant bulb pieces were defatted with n-hexane before extraction of total phenols(TP) and total alkaloids(TA) from the plant. Results showed that the plant contains about 0.95g/140g bulb as total phenols residue and 0.7g/150g bulb as total alkaloids residue. The major Tp compounds observed in HPLC assay were Salycilic acid, Sinapic acid, vanillic acid, Caffeic acid, Chlorogenic acid, and p-coumaric acid in desending quantity. Four layer had been collected that might contain different kinds of alkloids. The methanolic layer (1) and layer (3) which represented the chloroform layer after acidification represented the richest layers in 'Glanthamine' alkloid represented by HPLC assay. The antibacterial as inhibitory effects of TP and TA on *E.coli*, *P.auroginosa*, *S.aureus* and *Bacillus subtillus*, showed moderate antibacterial activity for both plant extracts in coparisin to some trade antibiotics.

Key words: *Narcissus tazetta*, Amaryllidaceae family, Total phenols,Total flavonoids.

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Introduction

Medicinal properties of plants belong to Amaryllidaceae family were already known in the fourth century B.C., when Hippocrates used its oil for the treatment of uterine tumors(Miroslav et al.,2015). This family contains many plants with important biological effects among them is the plant *Narcissus tazetta* which is known worldwide for being a good source for active constituents like alkaloids and polyphenols had been used for treatment many disorders that had risen now a day as Alzheimer disease (Tomasz and Jacek ,2009), thus the plant inhibit the enzyme cholinesterase responsible for Alzheimer condition development.(Al-Snafi,2020). The plant is spring-flowering plants, and rich with biological active components; among them; cardio active glycosides, different types of flavonoids, tannins, volatile oil, beside, steroids, terpenoids, alkaloids as well as anthraquinones (Kamenetsky and Okabo ,2012). **Phenolic compounds** as a secondary metabolites are produced in almost all plant kingdom via shikimic acid pathway (Talapatra and Talapatra,2015) expressed potential in health benefits as antioxidant(Fu et al.,2016), antifungal(Hammoda,2018), antiviral(Uthirapathy, 2021), anti-bacterial, cardio-protective(Chiu et al.,1992) and antitumor active agent(Ooi et al.,2000).

. In spite of their wide distribution in the plant kingdom, researchers have directed their attention to the health benefits of phenolic compounds and alkaloids (Dai and Mumper, 2010). Multi-drug resistance espically for antibiotics used today makes the infectious diseases more complicated for getting difficult to cure gave an importance towards new compounds from nature to overcome such problems***. Phenolic compounds and alkaloids extracted from the bulbs of Narcissus tazetta cultivated in Iraq were used in the current study to investigate the antibacterial activity represent the main aim of the work.***

Materials and methods:

Plant collection

After fresh collection of *Narcissus tazetta* bulbs from botanical garden at Baghdad city, steps for cleaning and dust removing were proceded to prepare the plant material for subsequent experiments.

Plant total phenolic and total alkaloids Extraction

i-Total Phenolic Extraction

A defatting process was done before phenolic compounds extraction starting from 350 g fresh bulb pieces using n-hexan as good non-polar solvent for removing any fatty materials. The resultant weight of defatted bulb was 140 g. to be macerated with 80% ethanol and left for 7 days in cool dark place. A process of filtration and drying were done and the residue was weighted and kept in dark container for further tests (Tonisi, 2020).

ii-Extraction of Total alkaloids: (Balasundram et al.,2006)

About 300 gm of fresh bulbs were soaked in normal hexane for 4 days, then filtered and discarded the filtrate to get defatting plant material that allowed to dry in room temperature for solvent residual removal. The dried defatting bulbs were weighted to get 150 g, and the following steps were taken on them:

1- Soaking with 500 ml of absolute ethanol alcohol with gentle heating to a temperature of 60 degrees Celsius for an hour with stirring, the bulbs were left soaked for another 24 hours at room temperature to ensure the extraction of almost all Alkaloids in the bub. The extract was filtered and allowed to dry and weighed ,then coded as sample No. (1)

2 - The bulb residue from step 1 was re-soaked in an acidified solution with a concentration of (5% HCL) 800 ml to reach the pH (pH = 2), the soaking was left for 4 days. Then it was filtered to show a white precipitate on the filter paper, and this salt precipitate was called the sample (2). After filtration, the acid filtrate was transferred to a separating funnel, then chloroform was added to it in a ratio of 1:1, the funnel was shaken gently. The two acidified aqueous layers and the chloroform layer were separated, to produce an upper acidified aqueous layer that was used in the next step. The lower chloroform layer was dried and called the dry chloroform layer after adding the acid to the sample (3)

3- The acidic aqueous layer was taken in the previous step and a base was added with a concentration of 10% Na2Co3 sodium carbonate to raise the pH value 10 to make the solution basic and then transferred to the separating funnel again and chloroform was added at a ratio of

1:1 to the solution and shaken gently. The second layer: the chloroform layer was dried. The dry layer of chloroform was named after the addition of the base sample No. (4).

All the dry layers were individually weighed and noted, and alkaloid detection was carried out using Dragendorff reagent for all layers and samples.

Samples positive for Dragendorff reagent were collected and the collected weights represent the total alkaloids present in the bulbs.

Determination of Total Phenols and Total Alkaloids by High Performance Liquid Chromatography: HPLC: (Ana-Maria et al.,2020, Özlem et al.,2019)

Table -1 showed the HPLC conditions for determination of total phenols and total alkaloids in plant bulb extracts with corresponding to standard and concentration for each

Table -1 HPLC conditions for phenols and alkaloids present in plant bulb extracts with corresponding to standard and their concentrations

HPLC Conditions	For Total phenols	For Total Alkaloids
Column	ODS L18 (10X 4.6Id) mm, 5µm particle size	ODS c18 (150*4.6 Id) mm 5mm partical site
Flow rate	0.7ml/min.	1 ml/min
Wave length /Detector	280 n.m/ uv-vis	280 n.m/ uv-vis
Tepreture		35 C
Mobile Phase	A=1% Acetic acid and **B**=Methanol 10%	**A**: 30 mmol /L Ammonium Bicarbonate + 0.7% Amonia Solution + 0.1% Tri ethyl amine **B**: Acetonitnle **A/B**: 30%
Standard (concentration)	vanillic acid(5µg/ml) Caffeic acid(5µg/ml) p-coumaric acid (5µg/ml) Chlorogenic acid (5µg/ml) Sinapic acid (5µg/ml) Salycilic acid(5µg/ml)	Glanthamine(6mg/ml)
Plant Extract(concentration)	12.5mg/ml	12.5mg/ml

Antibacterial Activity for Total Phenols (TP) and Total (TA)Alkaloids :

The determining Inhibitory Effect by well agar diffusion method of for Total Phenols and Total Alkaloids had been conducted as follow(Silva et al.,1987 ؛ Rayhana et al.,2020)*:*

A culture of *E.coli*, *P.auroginosa*, *S.aureus* and *Bacillus subtillus* previously grown in nutrient broth was streaked on Muller-Hinton agar, and then incubated under aerobic conditions at 37˚C for 24 hr. After incubation a cock porer (5mm) was used to withdraw discs of each plant extract and some antibiotic disc then put on surface of the Muller-Hinton agar that was inoculated (before) with 0.1 ml of pathogenic bacteria. After incubate, at 37˚C for 24 hr, the inhibition zone around the disc was estimated in (mm).

The determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for total phenols and total alkaloids*, (AL-Saadi,2016)*:

The MIC test demonstrates the lowest level of antimicrobial agent that greatly inhibits growth, the MBC demonstrates the lowest level of antimicrobial agent resulting in kill microorganism. Different concentrations of each plant extract were made in tubes containing sterile nutrient broth each. The concentrations were (1/9, 2/8, 3/7, 4/6, 5/5, 6/4, 7/3, 8/2 and 9/1) giving final volume 10ml in each tubes. Then each concentration was inoculated by 0.1ml culture previously grown in nutrient agar *E.coli*, *P.auroginosa*, *S.aureus* and incubated at 37 ˚C for 24 hr. After incubation the growth of tubes was observed and minimum inhibitory concentration and was determined as the lower concentration of the filtrate that gave no growth of each bacteria in the tubes, and Minimum Bactericidal Concentration MBC demonstrates the lowest level of antimicrobial agent resulting in kill pathogenic bacteria.

Results and Discusion

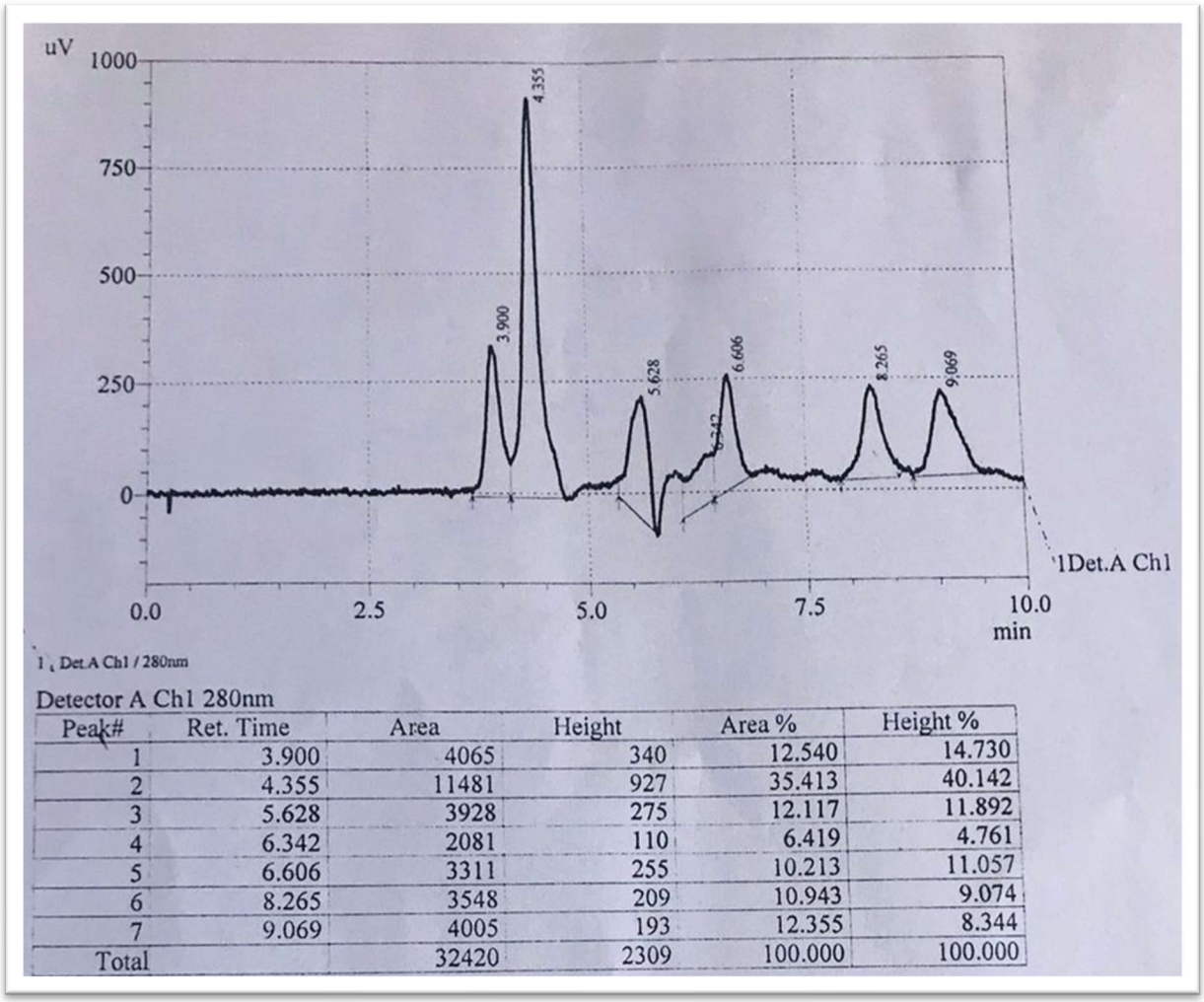
Plant total phenolic and total alkaloids yields from Extraction

The total phenol residue weight was 0.9544g/140g defatted bulbs. While the residue for total alkaloids extracted from bulbs were estimated by the summation of the weight of all layers gave positive results with dragendroff's test and that equal to about 0.7 g/150g defatted bulbs.

Determination of Total Phenols and Total Alkaloids by High Performance Liquid Chromatography: HPLC:

Figure (1) and (2) investigated the major phenolic compounds in the standard and sample extracted solution respectively. Table (2) showed the major phenolic compounds and their concentration detected in the plant bulb.

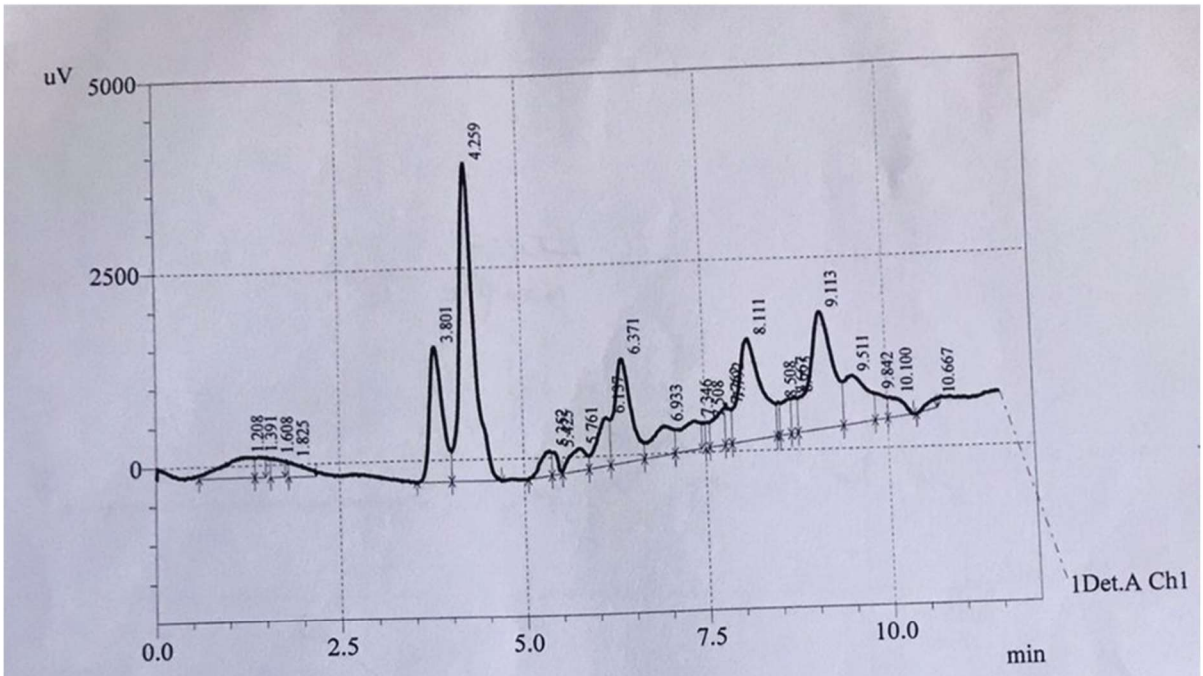
Figure (3) and (4) represented the HPLC chromatogram for the standard alkaloid 'Glanthamin' and the defatted bulbs.



Detector A Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.900	4065	340	12.540	14.730
2	4.355	11481	927	35.413	40.142
3	5.628	3928	275	12.117	11.892
4	6.342	2081	110	6.419	4.761
5	6.606	3311	255	10.213	11.057
6	8.265	3548	209	10.943	9.074
7	9.069	4005	193	12.355	8.344
Total		32420	2309	100.000	100.000

Figure (1) HPLC chromatogram for standard phenolic compounds



1 Det.A Ch1 / 280nm

Detector A Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.208	8102	269	3.294	1.548
2	1.391	2333	266	0.948	1.530
3	1.608	2684	241	1.091	1.391
4	1.825	2831	191	1.151	1.102
5	3.801	21312	1746	8.664	10.060
6	4.259	55293	4148	22.478	23.903
7	5.352	3531	323	1.435	1.862
8	5.425	1543	293	0.627	1.691
9	5.761	4647	287	1.889	1.656
10	6.137	7575	619	3.079	3.564
11	6.371	20710	1346	8.419	7.757
12	6.933	7606	375	3.092	2.161
13	7.346	7271	370	2.956	2.132
14	7.508	1138	329	0.463	1.896
15	7.762	5091	473	2.070	2.726
16	7.792	2523	521	1.026	3.001
17	8.111	28843	1324	11.725	7.631
18	8.508	1067	433	0.434	2.497
19	8.667	4148	458	1.686	2.641
20	8.725	2504	465	1.018	2.677
21	9.113	33813	1527	13.746	8.797
22	9.511	13321	628	5.416	3.616
23	9.842	3241	346	1.318	1.993
24	10.100	3525	250	1.433	1.440
25	10.667	1333	126	0.542	0.728
Total		245985	17354	100.000	100.000

Figure (2) HPLC chromatogram for plant extracted phenolic compounds

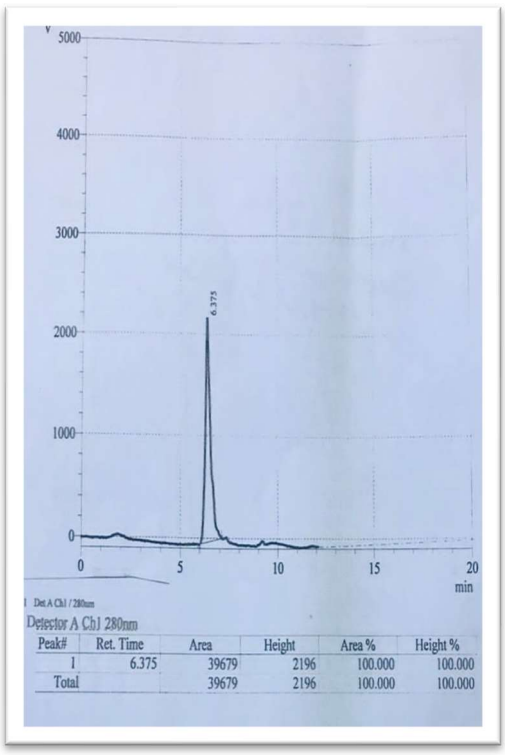
Table (2) HPLC analysis results for standards and the extracted phenolic compounds

Phenolic compound	Conc. µg / ml	Rt.in minutes For Standarad phenols	Rt.in minutes For the extracted phenols	Concentration µg / g plant
vanillic acid	**5**	**3.900**	**3.801**	**26.2**
Caffeic acid	**5**	**4.355**	**4.259**	**24**
p-coumaric acid	**5**	**5.628**	**5.761**	**6**
Chlorogenic acid	**5**	**6.606**	**6.933**	**11.5**
Sinapic acid	**5**	**8.265**	**8.111**	**40.6**
Salycilic acid	**5**	**9.069**	**9.113**	**42.2**

The plant bulb seemed to be good source of several phenolic compound, the most abundant was salycilic acid(42.2 µg / g) and sinapic acid(40.6 µg / g), with less extend vanillic acid (26.2 µg/g), caffeic acid(24 µg /g), chloragenic acid (11.5 µg/g) and the least content was p-coumaric acid(6 µg/g).

The HPLC chromatogram for alkaloids in the four extracted layer from plant bulbs were represented in the figures(3) and table (3) in respect with standard alkaloid "Glanthamine".

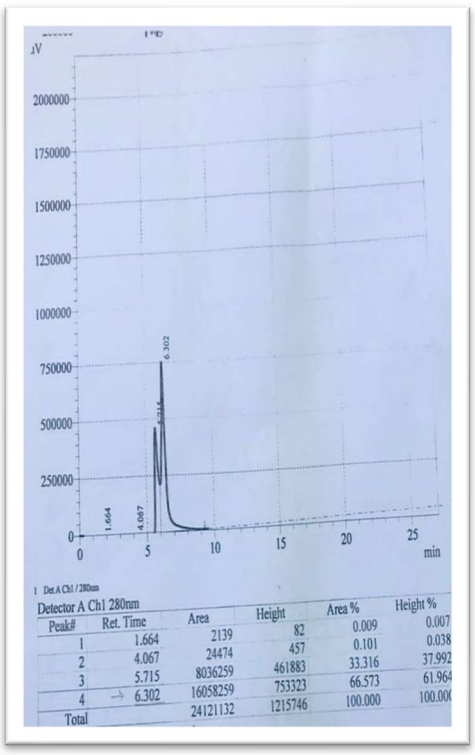
A few studies on the natural compounds present in the narcissus plant grown in Iraq and its biological activity against microbes, infections or various diseases had been conducted (Bushra and Mahmood, 2018). The phytochemical constituents and their concentration for this plant was employed in the current study differ from what is found in *Bati* and co. study (Bati et al.,2018)



Detector A Ch1 280nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.375	39679	2196	100.000	100.000
Total		39679	2196	100.000	100.000

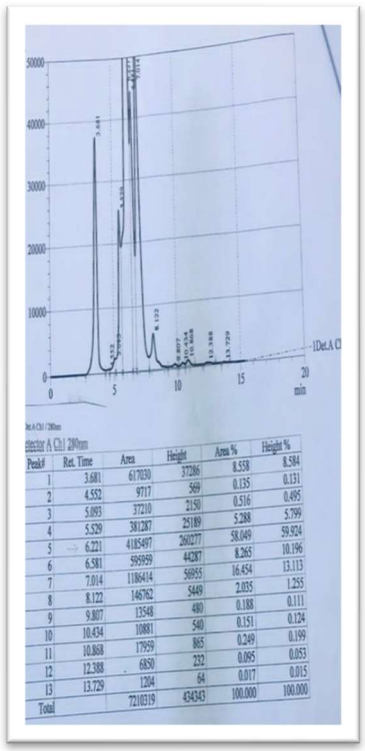
Glanthamine standard alkaloid



Detector A Ch1 280nm

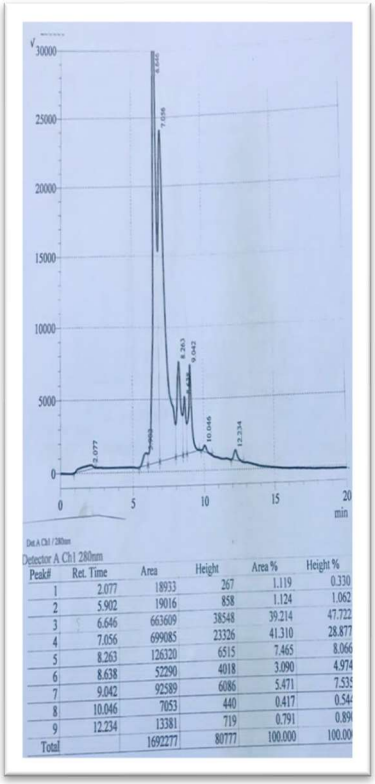
Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.664	2139	82	0.009	0.007
2	4.067	24474	457	0.101	0.038
3	5.715	8036259	461883	33.316	37.992
4	6.302	16058259	753323	66.573	61.964
Total		24121132	1215746	100.000	100.000

Layer 1



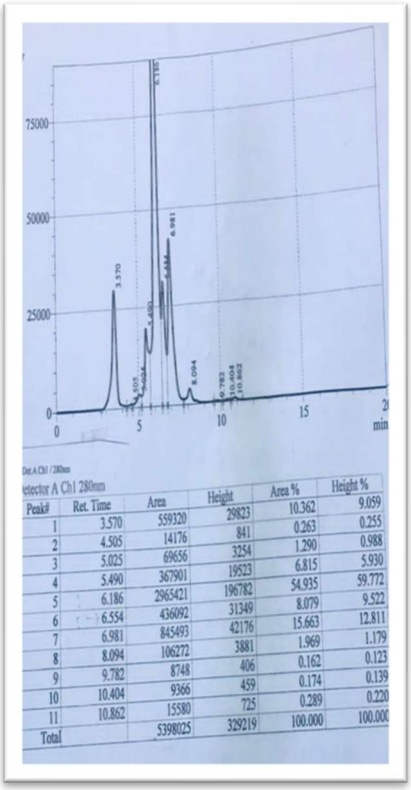
Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.681	617030	37286	8.558	8.584
2	4.552	9717	569	0.135	0.131
3	5.093	37210	2150	0.516	0.495
4	5.529	381287	25189	5.288	5.799
5	6.221	4185497	260277	58.049	59.924
6	6.581	595959	44287	8.265	10.196
7	7.014	1186414	56955	16.454	13.113
8	8.122	146762	5449	2.035	1.255
9	9.807	13548	480	0.188	0.111
10	10.434	10881	540	0.151	0.124
11	10.868	17959	865	0.249	0.199
12	12.388	6850	232	0.095	0.053
13	13.729	1204	64	0.017	0.015
Total		7210319	434343	100.000	100.000

Layer 2



Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.077	18933	267	1.119	0.330
2	5.902	19016	858	1.124	1.062
3	6.646	663609	38548	39.214	47.722
4	7.056	699085	23326	41.310	28.877
5	8.263	126320	6515	7.465	8.066
6	8.638	52290	4018	3.090	4.974
7	9.042	92589	6086	5.471	7.535
8	10.046	7053	440	0.417	0.544
9	12.234	13381	719	0.791	0.890
Total		1692277	80777	100.000	100.000

Layer 3



Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.570	559320	29823	10.362	9.059
2	4.505	14176	841	0.263	0.255
3	5.025	69656	3254	1.290	0.988
4	5.490	367901	19523	6.815	5.930
5	6.186	2965421	196782	54.935	59.772
6	6.554	436092	31349	8.079	9.522
7	6.981	845493	42176	15.663	12.811
8	8.094	106272	3881	1.969	1.179
9	9.782	8748	406	0.162	0.123
10	10.404	9366	459	0.174	0.139
11	10.862	15580	725	0.289	0.220
Total		5398025	329219	100.000	100.000

Layer 4

Figure (3) HPLC chromatogram for Glanthamine alkaloid standard and plant extracted Alkaloids in four layers (1,2,3,4)

Table (3) HPLC analysis results for standards and the extracted Alkaloids in four layers

Alkaloid / Layer	Area under the curve	Rt.in minutes	Concentration "Glanthamine" mg / ml
Glanthamine standard	**39679**	**6.375**	**0.006**
Layer1	**16058259**	**6.302**	**2.43**
Layer2	**Not found**	**-------**	**-------**
Layer3	**4185497**	**6.221**	**0.633**
Layer4	**436092**	**6.554**	**0.066**

Three layers appeared to contain Glanthamine alkaloid. Layers 1 and 3 were superior in containing the largest amount of the alkaloid mentioned

Antibacterial activity for Total Phenols and Total Alkaloids:

Determining inhibitory effects of TP and TA on *E.coli*, *P.auroginosa*, *S.aureus* and *Bacillus subtillus* in comparison to trade antibiotic by well agar diffusion method were shown in table(4), while figures(4),(5),(6), and (7), declare the zones shapes and diameters represented the inhibitory effects of TP,TA, and each extracted alkaloid layer toward the four pathogenic bacteria used in this study. In spite that layer 2 contained trace amounts of Alkaloid as shown in Dragendroff test or almost free from alkaloids as not detected by HPLC assay, this layer was employed for antibacterial assay.

Table(4)The inhibitory effect as inhibition zone of TP and TA on the four pathogenic bacteria in comparison to some antibiotic and their concentrations

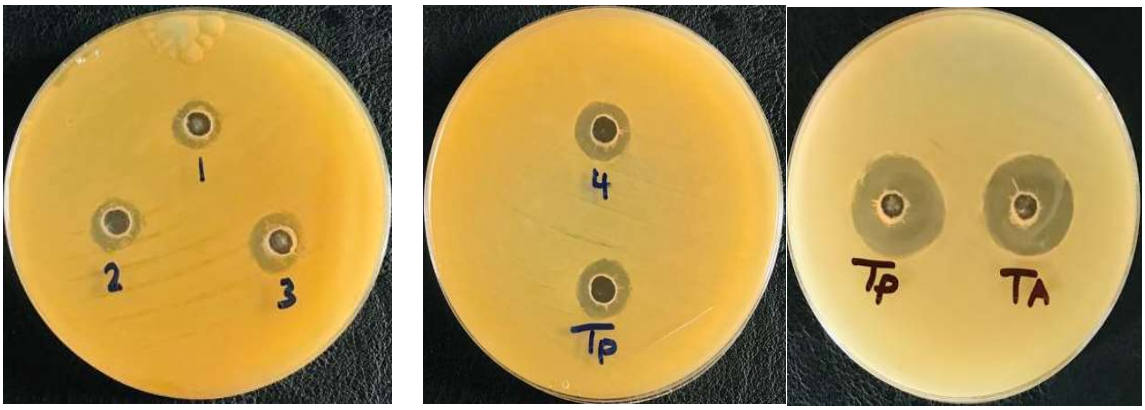
	Inhibition Zones (mm)				
Plant Extract or Antibiotic disc	Conc. (mg/ml)	*E.coli*	*P.auroginosa*	*S.aureus*	*Bacillus subtillus*
Total phenol(TP)	8	17	17	21	18
Total Alkaloid(TA)	8	16	20	23	22
Alkaloid Layer 1	8	13	11	12	13
Alkaloid Layer 2	8	11	16	13	14
Alkaloid Layer 3	8	12	12	15	14
Alkaloid Layer 4	8	19	11	14	25
TE-antibiotic	0.03	10	10	12	13
CTX-antibiotic	0.03	13	13	15	15
AX-antibiotic	0.025	10	13	13	13

IPM-antibiotic	0.01	20	20	15	20
AK-antibiotic	0.01	9	15	15	15
CIP-antibiotic	0.01	25	26	30	29

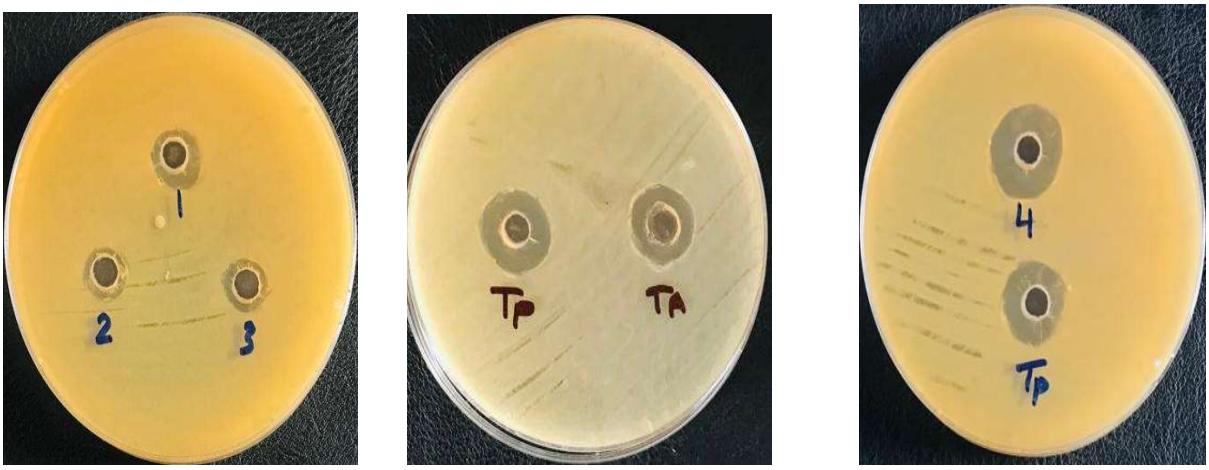
Since the discovery of penicillin, researchers conducted their affords toward finding other natural products with promising to be new drug candidates in treating antibiotic-resistant infections specially in multi resistance pathogens(Chee et al.,2021, Benedec et al.,2018).

Phenolic Compounds extracted from different plants play important role in killing and inhibiting the harmful tinny microorganism as these natural substance known to have antioxidant potency due to the hydroxyl group in their structure.(Aziz et al.,2022),

In general; total alkaloids (TA) appeared to be more potent than Total phenolic(TP) compounds extracted from plant bulbs. The different types of alkaloids extracted in each layer (except layer 2) affected the pathogenic bacteria in different manner. Table (4) declared that layer 4 possessed the potent antimicrobial effects as the of the antibiotic "imperamine"(IPM) activity.



Figure(4) The inhibitory zones diameters against *Staphylococus.aureus* byTP,and TAwith four extracted layers



Figure(5) The inhibitory zones diameters against *Escherichia coli* by TP,and TA with four extracted layers

Figure(6) The inhibitory zones diameters against *Bacillus subtillus* by TP,and TA with four extracted layers

Figure(7) The inhibitory zones diameters against *P.auroginosa* by TP,and TA with four extracted layers

For the determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the total phenols (TP) and total alkaloid(TA) for the plant extract, table(4) and(5) investigate the MIC and MBC value respectively for the three pathogenic bacteria used in this study

Table (5)The Minimum Inhibitory Concentration for TP and TA Against *Staphylococus.aureus, Escherichia coli and P.auroginosa*

Plant Extract	**MIC value(µg/ml) against *S.aureus***	**MIC value(µg/ml) against *E.coli***	**MIC value(µg/ml) against *P.auroginosa***
TP	5.04	5.04	4.032
TA	4.032	4.032	3.6288

Table (6)The Minimum Bactericidal Concentration for TP and TA Against *Staphylococus.aureus, Escherichia coli and P.auroginosa*

Plant Extract	MBC value(µg/ml) against *S.aureus*	MBC value(µg/ml) against *E.coli*	MBC value(µg/ml) against *P.auroginosa*
TP	7.2	7.2	5.04
TA	5.04	5.04	4.032

The inhibitory concentration (MIC) for total phenols(TP) extracted from the bulbs against ***S.aureus*** and ***E.coli*** represented by the concentration of 1.44 µg/ml. while for ***P.auroginosa*** bacteria MIC appeared at 0.288 µg/ml.. For the bactericidal minimum concentration (MBC) were 4.8 µg/ml for ***S.aureus*** and ***E.coli*** and 1.44 µg/ml ***P.auroginosa*** bacteria. In case of total Alkaloids(TA), The MIC against ***S.aureus*** and ***E.coli*** showed at 0.288 µg/ml which was more potent effect than TP even against ***P.auroginosa*** the minimum inhibitoy concentration was 0.0288 µg/ml. The total alkaloid(TA) MBC against ***S.aureus*** and ***E.coli*** was 1.44 µg/ml and 0.288 µg/ml against ***P.auroginosa***.

Conclusion:

Narcissus tazetta bulbs cultivated in Iraq considered as good source for phenolic compounds either as simple phenolic or flavonoids, besides their alkaloids rich components. The major Tp compounds observed in HPLC assay were Salycilic acid, Sinapic acid, vanillic acid, and Caffeic acid. Different alkaloids had been extracted and the famous is the Glanthamine alkaloid which was extracted in the acidified chloroform layer represented the richest layers in this alkaloid by HPLC assay. The antibacterial effects of TP and TA against *E.coli, P.auroginosa, S.aureus* and *Bacillus subtillus*, showed moderate antibacterial activity for both plant extracts in comparison to some trade antibiotics, that will encourage researchers to high light and conduct more efforts about investigating the plant active compounds of other medical or even prophylaxis effect.

Acknowledgment

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