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A Comparison between Total Phenols and Total Alkaloids Antibacterial Activity Extracted from Bulbs of *Narcissus tazetta* L. [#]

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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 as antimicrobial; 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 ethanolic layer (1) and layer (3) which represented the chloroform layer after acidification that represented the richest layers in 'Glanthamine' alkloid detected 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 comparison to some trade antibiotics.

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

مقارنة التأثير مضاد للجراثيم للمركبات الفينولية الكلية والقلويدات الكلية المستخلصة من بصيلات النرجس المستزرع بالعراق[#]

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الخلاصة

من المعروف أن نبات النرجس *Narcissus tazetta* من العائلة Amaryllidaceae غني بمركبات ايض فعالة حيويا مثل القلويات والفينولات والفلافونويد التي تم اكتشافها في اغلب أنواع هذه العائلة . ان نبات النرجس المستزرع في العراق ، لم يتم دراسة مكوناتها الفعالة كمضادة للميكروبات. ولذا كانت الدراسة الحالية متضمنة الفعالية ضد البكتيريا للمركبات الفينولية الكلية والقلويدات الكلية المستخرجة من هذا النبات. تمت إزالة الدهون من قطع البصيلات للنبات باستخدام n-hexane قبل عملية استخلاص الفينولات الكلية (TP) والقلويدات الكلية (TA) من النبات. أوضحت النتائج أن النبات يحتوي على حوالي ٠,٩٥ غم / ١٤٠ غم من البصيلات كمردود الفينولات الكلية (TP)المستخلصة و ٠,٧ غم / ١٥٠ غم من بصيلات النبات كمردود القلويدات الكلية(TA). كانت اهم مركبات Tp التي لوحظت في اختبار HPLC هي حمض الساليسيليك ، وحمض السينابيك ، وحمض الفانيليك ، وحمض الكافيين ، وحمض الكلوروجينيك ، وحمض الكوماريك بالكمية النهائية. تم جمع أربع طبقات قد تحتوي على أنواع مختلفة من القلويدات. كانت الطبقة الايثانولية (١) والطبقة (٣) التي تمثل طبقة الكلوروفورم بعد اضافة الحامض أغنى الطبقات في قلويد الجلانثامين نتج في اختبارتقنية الفصل الملون عالي الكفاءة HPLC . أظهرت التأثيرات المثبطة للجراثيم لـ TP و TA على E.coli ، و*P.auroginosa* و*S.aureus* وكذلك*Bacillus subtillus* فعالية متوسطة لكلا المستخلصين مقارنة بالمضادات الحيوية التقليدية.

الكلمات المفتاحية: بصيلات النرجس, الفصيلة النرجسية, الفينولات الكلية, الفلافونويدات الكلية.

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 [(1)]. 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 many disorders that had risen now a day as Alzheimer disease [(2)], thus the plant inhibits the enzyme cholinesterase responsible for Alzheimer condition development [(3)]. The plant is spring-flowering plants, and rich with biological active components; among them; cardio active glycosides, different types of flavonoids, tannins, volatile oil, besides, steroids, terpenoids, alkaloids as well as anthraquinones [(4)]. Phenolic compounds as secondary metabolites are produced in almost all plant kingdom via shikimic acid pathway [(5)] expressed potential in health benefits as antioxidant [(6)], antifungal [(7)], antiviral [(8)], anti-bacterial, cardio-protective [(9)] and antitumor active agent [(10)].

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In spite of their wide distribution in the plant kingdom, researchers have directed their attention to the health benefits of phenolic compounds and alkaloids [11]. 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. The plant was identified and authenticated by Prof. Dr. S. Abass -Department of Biology-College of Sciences- University of Baghdad to confirm all collective samples as *Narcissus tazetta* as follow**:**

Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Asparagales
Family	Amaryllidaceae
Genus	*Narcissus*
Species	*Tazetta*

Plant total phenolic and total alkaloids Extraction

A.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 a dark place at 25^0C. A process of filtration and drying were done and the residue was weighted and kept in dark container for further tests [12].

B.Extraction of Total alkaloids

About 300 gm of fresh bulbs were macerated with 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 done on them:

a. The defatted bulbs were macerated with 500 ml of absolute ethanol 99% with gentle heating up to temperature of 60 degrees Celsius according to reference13 with some modifications 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 Layer No. 1

b. The bulb residue from step 1 was macerated again in 800ml acidified aqueous solution with 5% HCL to reach the pH = 2, then left for 4 days, filtered and a white salty precipitate was appeared on the filter paper, and this salt precipitate was called , Layer No. 2. After filtration, the acid filtrate was transferred to a separating funnel, then chloroform was added in a ratio of 1:1, the funnel was shaken gently. Two layers were separated; the upper acidified aqueous layers and the chloroform layer, the acidified aqueous layer was used in the next step. The lower chloroform layer was dried and called the chloroform layer after adding the acid, Layer No.3

c. A base was added in a concentration of 10% Na_2Co_3 sodium carbonate to the acidic aqueous layer taken from the previous step to raise the pH value up to 10, then transferred to the separating funnel and chloroform was added with 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 as, Layer No. 4[13].

All the dried layers were individually weighed and alkaloid detection was carried out using Dragendorff reagent for all layers.

Samples gave positive result for Dragendorff reagent were collected. The summation of all layers weights represent the total alkaloids (TA) present in the bulbs.

Determination of Total Phenols and Total Alkaloids by High Performance Liquid Chromatography: HPLC [14],[15]

The HPLC conditions for determination of total phenols and total alkaloids in plant bulb extracts was shown in Table 1.

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
Temperature	Room temp.	35^0 C

Continued table 1.

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

The following equation was applied to calculate the concentration of each compound in the plant
Area under the curve for the sample/ Area under the curve for the standard X St.con X [vol.of extract/ wt.of the plant used.

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

Determining Inhibitory Effect by well agar diffusion method of for Total Phenols and Total Alkaloids had been conducted as follow [16],[17]

A culture of two types of G-ve bacteria *E.coli*, *P.auroginosa*, and two types of G+ve bacteria *S.aureus* and *Bacillus subtillus* **that** previously were 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).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for total phenols and total alkaloids18

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 Discussion

Plant total phenolic and total alkaloids yield from Extraction

Total phenol residue weight was 0.9544g/140g of 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.

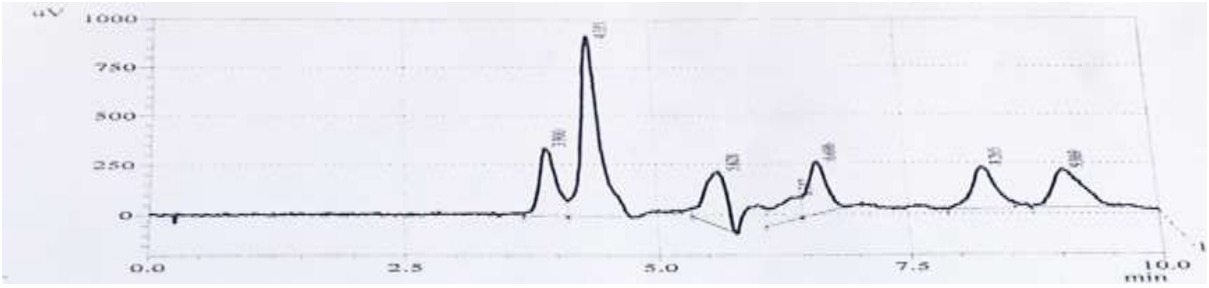


Figure 1. HPLC chromatogram for standard phenolic compounds.

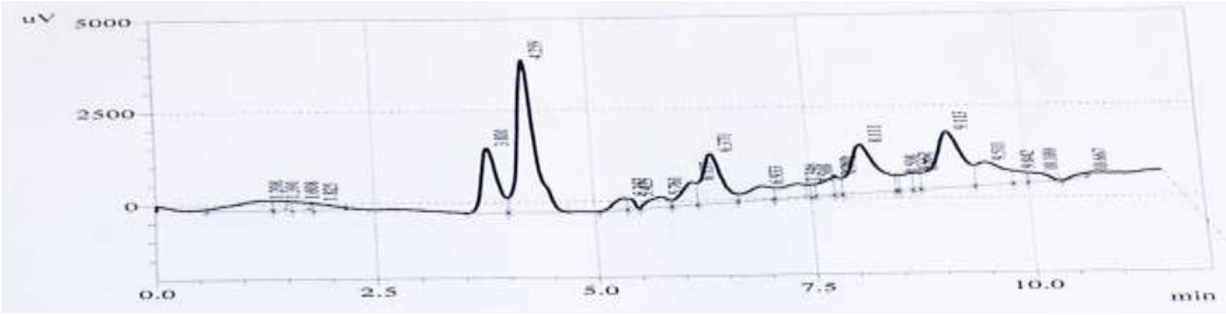


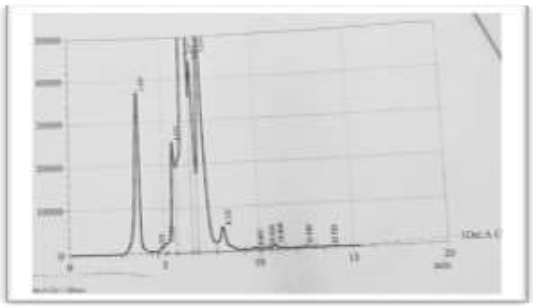
Figure 2. HPLC chromatogram for plant extracted phenolic compounds

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

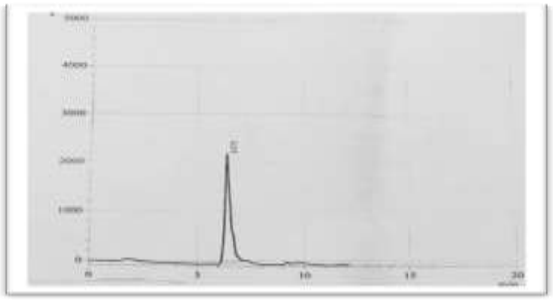
Phenolic compound	Conc. µg / ml	Rt.in minutes For Standarad phenols	Rt.in minutes For the extracted phenols	Concentration µg / g plant
vanillic acid	5	3.900	3.801	26.2
Caffeic acid	5	4.355	4.259	24
p-coumaric acid	5	5.628	5.761	6
Chlorogenic acid	5	6.606	6.933	11.5
Sinapic acid	5	8.265	8.111	40.6
Salycilic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

The plant bulb seemed to be a 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).

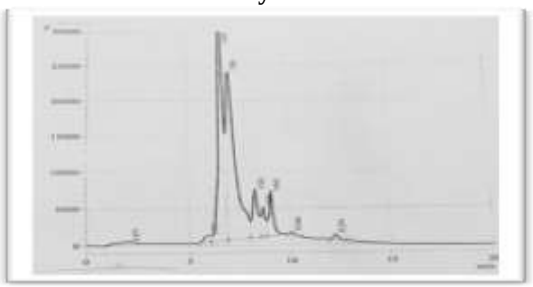
The HPLC chromatogram for alkaloids in the four extracted layer from plant bulbs were represented in the figures (3) and table (3) in respect with standard alkaloid "Glanthamine"



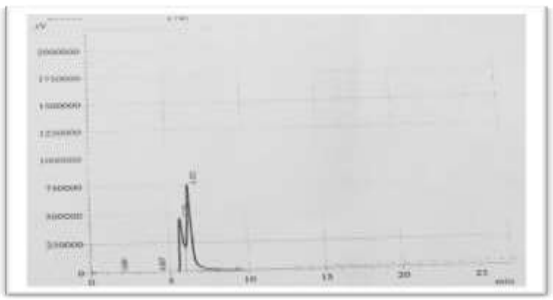
Layer 2



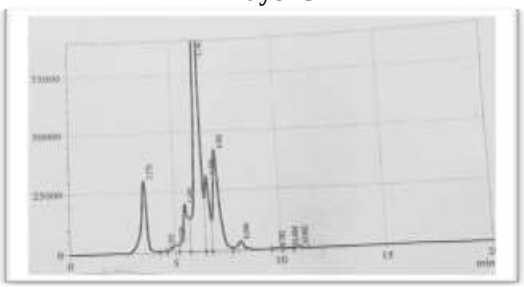
Glanthamine standard alkaloid



Layer 3



Layer 1



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 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 [19]. 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 [20]

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 with 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
30µg/ml Tetracycline TE-antibiotic	0.03	10	10	12	13
30µg/ml Cefotaxime CTX-antibiotic	0.03	13	13	15	15
25µg/ml Amoxicillin AX-antibiotic	0.025	10	13	13	13
10µg/ml Imipenem IPM-antibiotic	0.01	20	20	15	20
10µg/ml Amikacin AK-antibiotic	0.01	9	15	15	15
10µg/ml Ciprofloxacin 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 [21], [22].

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 [23] , [24].

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 [25,26]

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

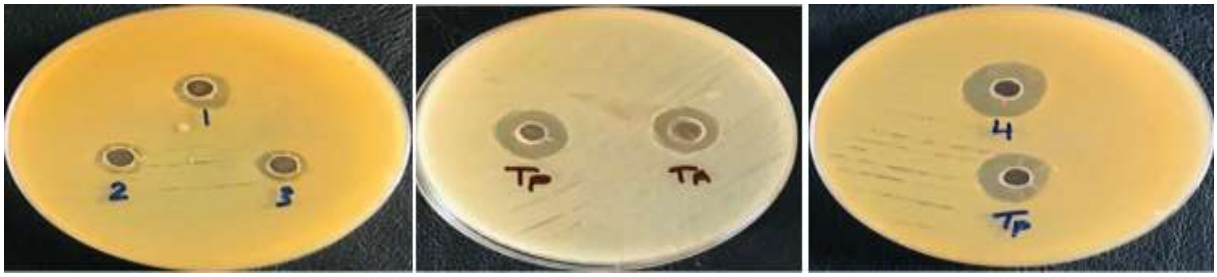


Figure 5. The inhibitory zones diameters against *Escherichia coli* by TP,and TA with four extracted layers.

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 5.04 µg/ml. while for P.auroginosa bacteria MIC appeared at 4.032 µg/ml.. For the bactericidal minimum concentration (MBC) were 7.2 µg/ml for S.aureus and E.coli and 5.04 µg/ml P.auroginosa bacteria. In case of total Alkaloids (TA), The MIC against S.aureus and E.coli showed at 4.032 µg/ml which was more potent effect than TP even against P.auroginosa the minimum inhibitory concentration was 3.6288 µg/ml. The total alkaloid (TA) MBC against S.aureus and E.coli was 5.04 µg/ml and 4.032 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.

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There is no conflict of interest regarding the publication of this manuscript.

Author Contribution

All authors confirm contribution to the paper as follows: study conception: Ola Kareem.; and the study design was by Zainab Yaseen and Arwa Abdul-Kareem ; data collection, analysis and interpretation of results by ; Ola Kareem, Arwa Abdul-Kareem and Zainab Yaseen. All authors reviewed the results and approved the final version of the manuscript.

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