IRAQI Academic Scientific Journals
العراقية المجلات الأكاديمية العلمية
ISSN:1813-1638
The Medical Journal of Tikrit University
Journal Homepage: http://mjtu.tu.edu.iq
MJTU
The Medical Journal of Tikrit University

Effect of punica granatum extract on growth of some serotypes of *Streptococcus pyogenes* isolated from acute and chronic cases of Tonsillitis in Children

[1]Heba Helal Moklof and [2]Raghad Saad. Ablkareem
[1]Deparetment Medical Laboratory Techniques, Kirkuk City, Kirkuk Governorate, Iraq
[2]Tikrit City ,Salah AL-Deen Governorte ,Iraq

*Corresponding author: E-mail: **raghadsaad @tu.edu.iq**

Received: 00/00/2024
Revising: 00/00/2024
Proofreading: 00/00/2024
Accepted: 00/00/2024
Available:online:31/12/2024

KEY WORDS:
Streptococcus pyogenes, Toncillitis,M12, punica granatum extract

ABSTRACT

Unregulated and frequent antibiotic use may cause microbial resistance in dangerous organisms. Thus, innovative synthetic and natural antibacterial compounds are necessary. In this perspective, plants provide most natural compounds. This study aimed to isolation and identification of *Streptococcus pyogenes* from acute and chronic cases and investigate the antibacterial properties of pomegranate peel extracts. The study included the collection of 215 samples (15 control and 200 patients from acute and chronic tonsillitis children), their age from(2 to 12 years) in kirkuk city during the period from (15-10-2023 to 1-2-2024) 140 (70%) showed no growth**.** A total of 200 clinical samples were obtained from patients. All samples were grown on blood agar and Azide blood agar and incubated aerobically at 37 °C for 24 hours. About 60 (30%) [40 chronic (20%) and 20 acute (10%)] of the samples showed positive for *Strep. pyogenes* growth. Three types of extracts of P. granatum pomegranate peels were used (acetone, ethanolic and aqueous extract). The aceton extract was more effective than ethanolic and watery , which inhibition zone were (29.3, 26.5, 14.55)mm**.** This study determined that the extracts from pomegranate peels had potent antibacterial properties against bacteria and have the potential to be utilized as medicinal treatment for human diseases caused by bacteria that are resistant to several drugs. The aceton extracts of pomegranate have higher antibacterial activity compared to the ethanolic and aqueous extracts.

DOI: http://doi.org/10.25130/mjotu.00.00.00

Pomegranate peel is a herbal drug; which are exactly opposite to microbial configuration of Tonsillitis. It is cheap and easily available to people, as it is best out of waste. With the intention of development of the pomegranate peel mouthwash, also Pomegranate is a superfood that is known for having high amounts of vitamin C and antioxidants that helps quickly recover without irritating already sore throat. Also, it is rich in many minerals and vitamins along with antioxidants that promote speedy recovery from tonsillitis in adults[6].Pomegranate peel extracts had a greater antibacterial effect on Gram-positive bacteria than on Gram-negative bacteria [7].

MATERIALS

215 samples—15 control and 200 patients—from children with acute and chronic tonsillitis, ages 2 to 12, were collected for the study between October 15, 2023, and January 1, 2024, at Kirkuk City (Kirkuk and Alnasr Hospital). 140 (or70%) did not grow. In cases of acute tonsillitis, samples were taken from the inside of the tonsils using a cotton swab; in cases of chronic tonsillitis, samples were taken from the inside of the tonsils after tonsillectomy. The tonsils were first sterilized by washing them in normal saline and then with 70% alcohol concentration. The tonsils were then opened using a sterile scalpel, and a smear of the fibrosis found in the tissue was taken using a disposable transport media swab. During collection, cotton swabs with the transport medium were utilized to guarantee the isolates' viability and long-term survival. Patients' consent was obtained before samples were collected, and their gender and age were noted. The samples that were gathered were immediately placed on Azide blood agar, blood agar, and β-Selective Streptococcus agar medium that included 5% fresh blood. These were then incubated at 37˚C for 24 hours with 5- 10% CO2.

DNA Extraction

INTRODUCT

Streptococcus pyogenes A common pathogen that causes infectious illnesses in children. It is the cause of up to 15 to 30 percent of pediatric cases of acute pharyngitis that happen in the age range of 5 to 15 years. can result in both suppurative and nonsuppurative illnesses, including glomerulonephritis, erysipelas, suppurative tonsillitis, scarlet fever, and rheumatic fever[1]**.** *Streptococcus pyogenes*, also known as S.pyogenes, is a species of aerotolerant, Gram-positive bacterium. These extracellular bacteria are composed of non-sporing, non-motile cocci that have a tendency to form chains. Since they are an uncommon but typically pathogenic component of the skin microbiota that can result in Group A streptococcal infection GAS [2].

Recently, there has been a growing interest in using medicinal plants and herbs as sources for producing medical drugs or as effective materials that can affect the composition of drugs. Various studies have been conducted to investigate the impact of plant extracts on the growth of microorganisms, suggesting their potential use in treating diseases caused by microbes[3].

Medical plants serve as a crucial resource for obtaining a wide range of therapeutic compounds, as recognized by the World Health Organization (WHO). Many medicinal plants have been utilized globally for the treatment of various ailments in everyday life [4]. The rise in antibiotic-resistant bacteria has necessitated the development of novel antimicrobial medications that are capable of effectively combating these bacteria. Furthermore, there has been significant interest in the utilization of medicinal plant components as an alternative form of medicine for the treatment of certain disorders. Additionally, specific chemicals derived from plant products have been precisely aimed at combating resistant bacteria[5].

Sterilization of extracts and preparation of dilutions:

For the purpose of using extracts in inhibition experiments, the method [9] was adopted in preparing and sterilizing the stock solution. 1 g of dry plant extract powder was taken and dissolved in 10 ml of sterile distilled water, so we had a stock solution with a concentration of 100 mg/ml. Sterilize the solution by filtration using filter papers (Whatman No.1) to get rid of the bacterial contaminants present in it and obtain a sterile storage solution. Use this solution as a source for preparing dilutions (75, 50, 25) mg/ml.

Qualitative and quantitative detection of chemical compounds using a high-performance liquid chromatography (HPLC) device:

This analysis was mentioned before [9] where a Phenomenex stationary phase column was used and the volume of particles is mM3 and the mobile phase is water: acetic acid with a flow speed of 1 ml/min. At a temperature of 30°C, using ultraviolet radiation at a wavelength of 245 nm, dissolving 0.1 g of the sample in 5 ml of an aqueous solution of methanol. The suspension was centrifuged at 7500 rpm for 15 minutes. The pure suspension was treated with charcoal to remove dyes, dried, and then resuspended in 0.1 ml/min of methanol for HPLC using a mixer. The mixture was passed through a disposable filter, then 20 microliters of the sample was injected into the HPLC system.

RESULTS

Detection of M12 serotype of *Strep. pyogenes*

The bacterial DNA amplified for this gene using PCR technique by used specific primers, and the optimum condition to amplified this gene in PCR shown in the Table (3-). The M12 protein confirmed by agarose gel electrophoresis, were the amplification to reveal a product of 410bp. The result showed that 80% of chronic and 75% of acute isolates carried M12 protein . Three types of extracts of P. granatum pomegranate peels were used (acetone,

Bacterial genomic DNA was extracted from bacterial isolates by using (Presto™ Mini gDNA Bacteria Kit) and done according to company instructions

Gel Electrophoresis

Amplicons were visualized on 1.5% Agarose gel by Electrophoresis. The PCR products were electrophoresed through agarose gel with current 5 V/CM^2 for about 45 min. Gels are photographed under UV light.

Plant samples

Samples of pomegranate fruits were collected at the neighborhood market, and after the peels were removed from the fruits, they were let to dry at room temperature for a week. Before being utilized, the peels were pulverized and kept in the refrigerator.

1. Preparation of Aqueous Extracts:

40 grams of the plant model were mixed with 160 ml of sterile distilled water, stirred the mixture using a shaker, and left the mixture in the refrigerator for 24 hours for soaking. It was then filtered using several layers of gauze to get rid of uncrushed plant parts and remaining fibers, then filtered again using a 0.45-diameter Millipore microfiltration unit to prevent the passage of germs from the filtrate. The mixture was then placed in an electric oven at a temperature of 40°C until all the water evaporated and the extract remained in the baker's hall. Then the extract was placed in glass bottles with a tight lid and kept frozen until used.

2. Preparation of alcoholic extracts:

- Preparation of ethanolic extract

The extract was prepared by the same previous method used in the aqueous extract, except that the solvent was replaced with ethyl alcohol at a concentration of 95% [8].

- Preparation of acetone extract

The extract was prepared in the same previous way as used in the ethyl extract, except that the solvent was replaced with acetone at a concentration of 80% [8].

granatum plant. This technology is characterized by high efficiency and accuracy in carrying out the quantitative and qualitative estimation of the medicinal compounds to be diagnosed through its ability To identify active compounds. The results of the HPLC examination that was conducted in this study showed the presence of the compound (Tannic acid , Elagic acid , Gallic acid Rutin, and Querictin.

The amount of dissolution is important and depends on the length of the reaction between the dissolved compounds and the stationary phase. Analysis using a high-performance liquid chromatography (HPLC) device, through which compounds were detected from the extract of pomegranate peels, and the retention time was determined. These compounds were determined based on the detention time and using standard solutions for these compounds, and the tannic compound was isolated acid in a holding time of (7.303 min), the compound Ellagic acid was isolated in a holding time of (4.32 min), the compound Gallic acid was isolated in a holding time of (8.5 min), the compound Rutin was isolated in a holding time of (4.586 min), and the compound qurtecin was isolated in a holding time of (4.236 min).

ethanolic and aqueous extract). Table (2) shows the diameters of inhibition for these extracts and the significant differences between the extracts. The aceton extract was more effective than ethanolic and watery , which inhibition zone were (29.3, 26.5, 14.55)mm.

The effect of the acetone extract on the S.pyogenes at different concentrations (25%, 50, 75, and 100)% . The highest diameter of inhibition were recorded in concentration 100% that was (24± 0.57mm), followed by concentration 75%, as recorded inhibition zone (22± 1 mm), the lowest inhibition zone recorded in concentration 25% and 50% that were (19±1.3and 19.3±1.5)mm respectively. As for the ethanolic extract, it was effective at the concentration of 100% the diameter of inhibition reached (23.8 ±2.1 mm), while at the concentration of 75%,50%,25% the diameter of inhibition was (19.8±1.74, 24. 19±1.88, 16±2.1), As for aquous concentration, the diameter of Streptococcus was recorded at concentration 100% ,75%,50%,25% as the diameter of inhibition were(15±0.86,14.7±0.98,14.5±0.81,14±1.2) mm.

High-performance liquid chromatography was used for the quantitative and qualitative diagnosis of some flavonoids present in the alcoholic extract of the ***P.***

Table 1 .Effect of P. granatum peel extract on *S. pyogenes.*

Type of extract	**Concentration**				**Average extract**
	25	٥٠	٧٥	١٠٠	
Acetone	19±1.3 b	19.3±1.5 b	22± 1 ab	24± 0.57 a	29.3 A
Ethanol	16±2.1 c	19±1.88 b	19.8±1.74 b	23.8±2.1 a	26.5 B
Watery	14±1.2 a	14.5±0.8 1a	14.7±0.98 a	15±0.86 a	14.55 C

Table 2.Phenolic compounds isolated using HPLC technology from pomegranate peels

Sabstance	Detention time	Concentration (mg/ml)
Tannic acid	7.303 min	38.96
Elagic acid	4.32 min	1276.76
Gallic acid	8.5 min	2448.39
Querciten	4.586 min	29.318
Rutin	4.236 min	71240.15

Table 3.The Housekeeping gene PCR primers with their nucleotide sequence and product size(10)

Gene name	Primer Name	Sequence (5'-3')		Product Size
	M12	F	GCGTATAATGAGCTTAGCGC	410bps
		R	GCAAGTTGATGGCCTAATGC	

Abbreviations
GAS:groub A *streptococcus pyogenes*
WHO: world Health organization
Pcr:polymerase chain Reaction
DNA: deoxyribonucleic acide
UV: ultrviolet
HPLC: High-performance liquid chromatography

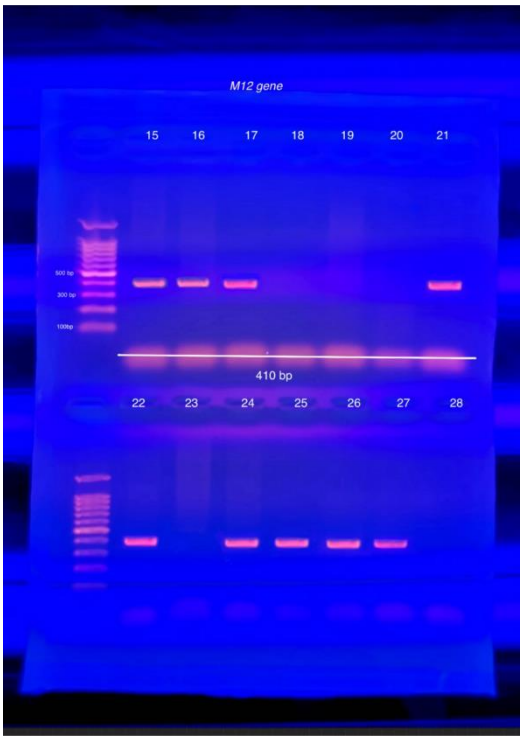

Figure 1.Conventional PCR for detection of M12 gene (bp), in *Streptococcus pyogenes* isolates. PCR product the band size 410 bp. The product was electrophoresis on 1.5% agarose at 5 volt/cm^2. 1x TBE buffer for 45 minutes. N: DNA ladder (100).

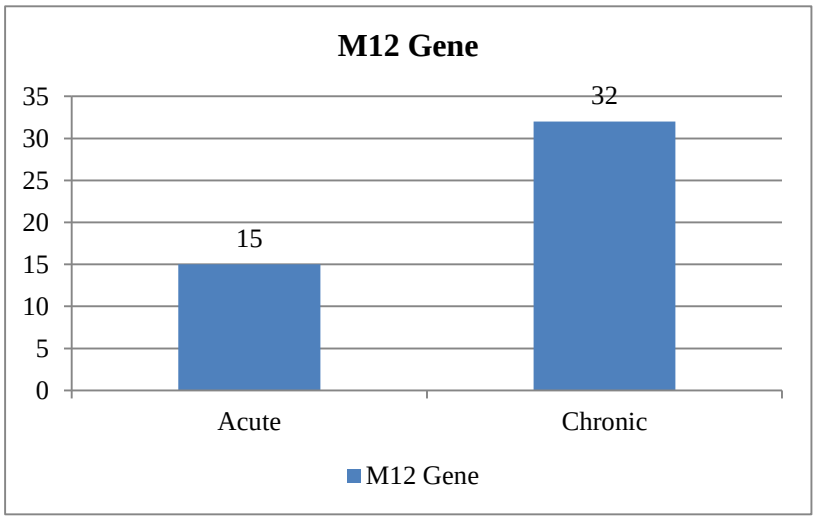

Figure 2.percentage of M12 Gene isolated from acute and chronic tonsillitis

exploration of novel antibacterial compounds derived from plants [15]. Extensive research has been conducted on the biological qualities of Punica granatum, and the findings indicate that this plant possesses significant medicinal properties [16]. This work demonstrated the antibacterial properties of pomegranate peel extracts obtained using acetone, methanol, and aqueous solvents against S.pyogenes. These results are consistent with other investigations. In their investigation, Al Hassnawi [17] found that both alcoholic and aqueous peel extracts of Punica granatum, at various doses, exhibited effectiveness against all tested microorganisms. Furthermore, the findings were consistent with Haider [18] and Al-Zoreky [6], who reported that the alcoholic extract of peels exhibited significant inhibitory zones against eleven microorganisms in their investigation. The antibacterial action of Punica granatum fruit rind is likely attributed to the presence of hydrolysable tannins and polyphenolics, including punicalagin and gallagic acid. These compounds, even at low concentrations, exhibit the most antibacterial effects against the tested strains[19]. The study done by [20] showed that bacteria were inhibited by the methanol extract and in some cases methanol extract gave better antibacterial

DISCUSSION

The study carried out in AL –Diwaniyah province taken 300 samples from tonsillitis patients showed 165 positive bacterial growth and 135 with no growth [10]. The study of Karim, *et al*. showed positive bacterial growth (41.9%) of isolated bacteria from tonsillitis patient were *Strep. pyogenes* which was close to current study percentage [11]. *S. pyogenes M12* is most frequently isolated from scarlet fever cases that come as complication of strep. pyogenes chronic infection as showed in study of You *et al.*[12]It was found that Streptococcus pyogenes, namely serotype M12, produces a protein that may bind to human IgG3. The Western blot study of the partly purified M12 protein, when exposed to IgG3 myeloma protein, revealed that both the M12 antigen and the receptor protein had a same apparent size of [13]. The occurrence rate of M12 S pyogenes began to decrease from 2011, whereas M1 started to rise and eventually surpassed M12 in 2013 and 2014, coinciding with the escalation of scarlet fever cases. Since 2014, there has been a rise in the frequency of non-dominant kinds, with an increase of occurrences[14]. The rise in antibiotic resistance, coupled with the undesirable side effects of synthetic medications, has prompted significant interest in the

discrepancy in the effects of aqueous and alcohol pomegranate peel extracts. This may be due to the quality and quantity of the active substance released and dissolved, as alcohol has a higher ability to dissolve compared to water. This is because the active substance that affects germs dissolves in organic solvents more than it dissolves in water [27].The inhibitory effectiveness of pomegranate peels is due to them containing a number of effective compounds such as tannins, phenols, flavonoids, and alkaloids. Chemical analysis of the ethanolic extract of pomegranate peels also indicated that it contains sterols, flavonoids, triterpenes, phenols, and tannins [28], the latter of which is one of the important and well-known compounds In Its effectiveness against microbes. This is consistent with [29] who isolated these compounds from different plants, with the difference in retention time, which is due to the difference in the type of device used, the conditions under which the test was conducted, and the type of plant from which the compounds were isolated.

The inhibitory role is due to the action of some phenols as antioxidants because they have quenching properties and thus act as reducing agents or hydrogen donors and have the ability to quench free radicals [30]. Phenolic compounds also fundamentally affect the balance of cellular content through causing damage to some proteins. Found in the cell membrane, in addition, they work to denature proteins, as they are absorbed by the surfaces of proteins, thus forming a complex with them, changing the shape of the cell, leading to the cessation of its growth or death. As for flavonoids, they are important antioxidants due to their diverse mechanisms of action [31].

Conclusion

Our findings indicate that the extracts from pomegranate peels exhibit potent antibacterial properties against bacteria and have the potential to be utilized as medicinal treatment for human diseases activity than tetracycline. This may be due to the active compounds of plant extract can readily be dissolved or extracted in methanol that could be responsible for growth inhibition of these bacteria. It has been demonstrated that different phytoconstituents have different degree of solubility in different types of solvents depending on their polarity [21]. Other study by[22] showed synergistic effect were tested between all plants extracts and antibiotics Amoxicillin, Cephalexin, Erythromycin, Trimethoprim against ten gram positive bacteria isolates using disc diffusion method. Result showed synergism was verified for all the plant extracts and plant extract with Trimethoprim showed the highest synergism

This is a discrepancy in studies and a discrepancy in the biological effectiveness of plant extracts (aqueous and alcoholic) and is due to the type of plant extract, the type of bacteria tested, the method used in extraction, and the polarity of the solvent used [23]. From the results above, it is clear to us that the effect of the alcoholic extracts was highly effective compared to the aqueous extract, and it is similar to the results we reached regarding the effect of the extracts on yeast. The findings indicated that the methanol extract exhibited more efficacy against bacteria compared to the aqueous extract. The outcome Similarly, Ali et al. [24] reported that the methanolic extract of pomegranate fruit peels is more potent than the aqueous extract. Furthermore, it is logical to infer that the primary chemical components responsible for antibacterial effects were highly concentrated in the alcoholic fraction. Furthermore, this finding aligns with the research conducted by Ashwaq et al. [25], which demonstrates that the methanol extract exhibited a significantly higher inhibition rate compared to the aqueous extract against pathogenic bacteria. Contrary to the findings of Khan and Hanee[26], our study reveals a

Antibacterial Activity of Water, Ethanol and Methanol Extracts of Ocimum gratissimum, Vernonia amygdalina and Aframomum melegueta. Advance Application Science Research, 2012; 3: 844-848.

5- Vasudha, P.; Thangjam,R.C.; Rituparna, C.; Bangar, R.; Richard, L. and Mamatha, B. Evaluation of the antimicrobial activity of *Punica granatum* peel against the enteric pathogens: An *in vitro* study. Asian J. Plant Sci. Res., 2011; 1(2): 57-62.

6- AL-ZOREKY, N. S. Antimicrobial activity of pomegranate (Punica granatum L.) fruit peels. *International journal of food microbiology*, 2009, 134.3: 244-248. https://doi.org/10.1016/j.ijfoodmicro.2009.07.002

7- NAZIRI, Z.; RAJAIAN, H.; FIROUZI, R. Antibacterial effects of Iranian native sour and sweet pomegranate (Punica granatum) peel extracts against various pathogenic bacteria. 2012. https://doi.org/10.1016/j.ijfoodmicro.2009.07.002

8- Al-Joboory, A. and Al-Rawi, M. Natural pharmacology. 1st ed. Baghdad , dar Al-Huriah,1994 . DOI: 10.21608/JPP.2016.44281

9- Amani, T. Characterization of poly phenols in tunisian olive with anticancer capacity using liquid chromatography coupled to mass spectrometry , Doctoral thesis, university of Ezmir,2012.

10- Wajid A R & Abd AL-Mayahi F S. Molecular Identification of Streptococcus pyogenes isolated from tonsilitis in AL–Diwaniyah province.2023;12(3):2230-5807.

11- Karim GF, AL-Salihi SS, Atya QM, Abass KS. Aerobic and Anaerobic Bacteria in Tonsils of Different Ages with Recurrent Tonsillitis. *SCOPUS IJPHRD*

caused by bacterial infections that are resistant to several drugs. The aceton extracts of pomegranate have greater antibacterial activity compared to the ethanolic and aqueous extracts.

Conclusion of Interest

The benefit of pomegranate extracte for tonsillitis in children.

ACKNOWLEDGEMENTS

I would like to present my great gratitude to the head of the Department of Microbiology Professor Dr.Raghad Saad Ablkareem, for her encouragement, support, concern, and precious advice throughout the course of this thesis, and I wish to express my deep gratitude and sincere thanks for Professor Dr. Wisam Suhail Najim, the dean of college of medicine, and all teaching staff members of the kirkuk hospital and al-nasr hospital for their effort during my training period. I am particularly grateful to the healthcare workers and patients who participated in this study for their willingness to assist by giving their information.

REFERENCES

1- ts. OKABE, Toshinari, et al. Change during an 8-Year Period in Streptococcus Pyogenes emm Types in Pharyngeal Isolates from Children with Noninvasive Infections. *Journal of Nippon Medical School*, 2020, 87.4: 211-214. https://doi.org/10.1272/jnms.JNMS.2020_87-502

2- BROUWER, Stephan, et al. Pathogenesis, epidemiology and control of Group A Streptococcus infection. *Nature Reviews Microbiology*, 2023, 21.7: 431-447. https://doi.org/10.1038/s41579-023-00865-7

3- Majeed, G.R. and Al Shatti, S.M. Effect of antimicrobial activity of some plant extracts on some microbial growth. J.Vet. Sci., 2002; 8(2): 101-108.

4- Alo, M.N. ; Anyim, C.; Igwe, J.C.; Elom, M. and Uchenna, D.S.

Pharmaceutical Research, 2017, 6.8: 2426-36.

17- Haider.The effect of Some medicinal plant extracts against bacteria *Staphylococcus*,*Streptococcus*, *Escherichia coli*,and the Yeast *Candida albicans*, Cryptococcus.Di-khar journal.2008;Vol(3)2.ISSN 1991-8690.

18- Singh R.P., Chidambara Murthy K.N. and Jayaprakasha G.K. Studies on the antioxidant activity of pomegranate (Punica granatum) peel and seed extracts using in vitro models. J Agric Food Chem, 2002; 2: 50(1): 81-6. https://doi.org/10.1021/jf010865b

19- Reddy,M.K.; Gupta,S.K.; Jacob, M.R.; Khan,S.I. and Ferreira, D. Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from Punica granatum L. Planta Med, 2007; 73(5): 461-7. DOI: 10.1055/s-2007-967167

20- ISLAM, Soriful, et al. In vitro antibacterial activity of methanol seed extract of elettaria cardamomum (L.) maton. *Agriculturae Conspectus Scientificus*, 2010, 75.3: 113-117.

21- El-Mahmood A. M., Doughari J. H. Phytochemical screening and antibacterial evaluation of the leaf and root extracts of *Cassia alata* Linn. Afr J Pharm Pharmacol,2008, 2(7): 124-129

22- Naji, Semaa Ammar ., Banno, Ilham Saeed Abdul-Karim. Synergistic effects of plants extracts and antibiotics on bacteria *Staphylococcus aureus* isolated from oropharynx. Athesis. University of Baghdad College of Education. Ibn Al Haitham. 2012

CITATION SCORE.2019; *10*(9): 132..

12- You Y, Davies M R, Protani M, McIntyre L, Walker M J & Zhang J. Scarlet fever epidemic in China caused by Streptococcus pyogenes serotype M12: epidemiologic and molecular analysis. *EBioMedicine*,2018; *28*: 128-135. DOI:https://doi.org/10.1016/j.ebiom.2018.01.010

13- Alamiri F, André O, De S, Nordenfelt P & Hakansson A P. Role of serotype and virulence determinants of Streptococcus pyogenes biofilm bacteria in internalization and persistence in epithelial cells in vitro. *Frontiers in cellular and infection microbiology*,2023; *13*: 1146431. Volume 13 - 2023 | https://doi.org/10.3389/fcimb.2023.1146431

14- You Y, Peng X, Yang P, Wang Q & Zhang J. 8-year M type surveillance of Streptococcus pyogenes in China. *The Lancet Infectious Diseases*,2020; *20*(1): 24-25. DOI:https://doi.org/10.1016/S1473-3099(19)30694-2

15- Duman, A. D., Ozgen M., Dayisoylu, K. S., Erbil, N. and Durgac, C. “Antimicrobial activity of six pomegranate (Punica granatum L.) varieties and their relation to some of their pomological and phytonutrient characteristics., Molecules, 2009; 14(5): 1808–17. https://doi.org/10.3390/molecules14051808

16- AL HASSNAWI, A. A. Evaluation of antibacterial activity of aqueous and methanolic extracts of pomegranate peels (Punica Granatum Lin.) against some bacteria. *World Journal of*

Africa Journal of Ethnopharmacology, 2006; 103(19): 139-142.

28- Voravuthikunchai S, Lortheeranuwat A, Jeeju W, Sririrak T, Phongpaichit S,and Supawita T: Effective medicinal plants against Enterohaemorrhagic *E.coli* O157: H7 Journal of Ethnopharmacology .2004;94:49-54.

29- Kais K.K, and Mohamad, I.N Rami A.T.Extraction and identification of phenol compounds from Bitter Melon *Momordica charantia* fruits and their role as antioxidants. Journal of Biotechnology Research Center2013;. Vol.7 No.1.

30- Hakkim, F.L.;Arivazhagan, G. and Boopath, RAntioxidant property of selected *Ocimum* species and their secondary metabolite content. J. Med. Plan. Res. 2008;2: 250-25 .

31-Pietta , P. Flavonoids as antioxidanJ. Nat. Prod.2000; 63, 1035 – 1042**.**

23- Mitscher, L.A. ;Leu, R. ;Bathala, M.S. ;Beal, J.L. and White, RAntimicrobial agents from higher plants . lioydia .1972; 35:157-166 .

24- Ali S., Ahmad G, Ahmad M and Hassan R. Antimicrobial activity of aqueous and methanolic extracts of pomegranate fruit skin, Avicenna Journal of Phytomedicine Received, 2011; 1(2): 67-73.

25- Ashwaq, T., Hamad, N. and Ahmed, M. The inhibition activity of extract Punica granatum cortex on growth some pathogenic bacteria which isolate from human stomach and intestinal. Anbar University Journal of Sciences, 2009; 3(2).

26- Khan, J.A. and Hanee, S. Antimicrobial properties of Punica granatum peel. Int. J. Appl. Bio. and Pharm. Tech, 2011; 2(3): 23.

27- Buwa,L.V. and Staden, J.V. Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South