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Effect of some plant extracts (Syzygium aromaticum and Anthemis nobilis) against *Streptococcus pyogenes*

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Abstract

Streptococcal diseases are associated with respiratory tract infection that is a substantial reason of morbidity and mortality through the world**.** Plant extracts and other biological active compounds isolated from plants have increased wide spread interest for the treatment of disease since ancient time. This research amid to estimate the inhibitory activity of alcoholic extracts of *Syzygium aromaticum* and *Anthemis nobilis* against eight isolates of pathogenic bacteria *Streptococcus pyogenes* obtained from patients with respiratory tract infections who visiting Tikrit Teaching Hospital, and compare this antimicrobial activity with the activity of some antibiotic to inhibit bacterial growth using susceptibility test antibiotic. Antimicrobial activity was measured by using disc diffusion method. Result revealed that the all isolates more sensitive to alcoholic extract of *Syzygium aromaticum* at the concentration 100% and less sensitive to other concentrations, while the extract of *Anthemis nobilis* had no inhibition effect against all strains of *S. pyogenes*. Results of susceptibility test antibiotic revealed that all bacteria isolates were sensitive toCirpfloxacin, Amikacin, Gentamicin 100%, while all the isolates were resistant to other antibiotic at different rates (Trimethoprim/sulphamethoxazole 87.5%, Piperacillin 50%, Amoxicillin 75%, Meropenem 25%, and Ceftriaxone 12.5%). From these results we concluded that the inhibitory activity of alcoholic clove extract at 100% and 75% was comparable to that of to Cirpfloxacin, Amikacin, Gentamicin antibodies on the growth of these bacterial isolates.

Introduction:

The Gram- positive bacteria streptococci are facultative anaerobic, heterogeneous, spherical which occur in chain or in pairs. According to the significant virulence factor M protein which originate on the cell wall against the created of protective antibodies, was subdivided these bacteria to group A streptococci [1]. Streptococcal diseases are associated with respiratory tract infection (pharyngitis, tonsillitis), bloodstream, Arthritis, meningitis, neonatal sepsis and skin infection. These diseases are also a major reason of morbidity and mortality through the world [2,3]. *Streptococcus pyogenes* is distinguished as the group of

streptococci that able to infecting humans at greatest the upper respiratory tract through adhesion and colonization the mucosal surface epithelial cells of host [4]. this infection is widespread in school- age children ranged from 5 to 15 years, and around 600 million of pharyngitis and the skin infection about 100 million [5,6]. Antibiotic are generally used to decrease and avoid the negative effect of infections produced by opportunistic bacteria. However, the increase of bacterial multidrug resistance is due to the wrong and undiscerning usage. [7,8]. Furthermore*, S. pyogenes* forms biofilm, that was related with antibiotic treatment failures. Therefore, there is a critical need to find a new effective antibacterial and anti-biofilm agents which could find use in alternative healing for streptococcal pharyngitis. This situation stimulated researchers to find alternative antimicrobial agent, as the role of antibiotics to avoid the growing of pathogens [9].

Medicinal plant are the important natural resources that can be continually renewed and have an effective role in protective people from disease, it is also an important source for containing enormous amounts of antimicrobial agents. The extracts and other biologically active substances which isolated from plants have increased wide spread attention for the treatment of disease since ancient time due to the little side effect and well compatibility with the human body [10].

Syzygium aromaticum (Clove) belong to family Myrtaceae a rich basis of bioactive compound that considered as alternative solution to control the resistant of microbes. The active constituent of clove include: eugenol, chavicol, tannin, vitamin B, humulenes, and acetylsalicylate, phenol, oleic acid, isopropyl myristate, espintanol, caryophyllene oxide, n-hexadecanoic [11]. Hema et al., (2012) pointed that the ethanolic clove extract containe: octadecanoic acid, 9,12-octadecadienoic acid (Z, Z)-, eugenol, and Caryophyllene [12]. It has many medical advantages include antioxidant, anticancer, antiviral, antifungal, antiparasitic, antimutagenic, antithrombic, anti-inflammatory, and antidiabtic [13, 14] and has numerous uses like for diarrhea, vomting, cough, nausea, dyspepsia, flatulence, and stomach distention [15]. Several studies have confirmed effective antibacterial, antiviral, antifungal activities of clove [16, 17].

The medicinal plant *Anthemis nobilis* (Roman chamomile) is one of the most widespread among people and it is known as the gold flower, from the Asteraceae family, plants on all kinds of soil and it is unaffected by the cold. It is widely distributed all around the word, and the traditional used depending on the plant parts such as stem, flower, leave, and whole plant in addition to the preparation methods [18]. *A. nobilis* is thought to have anti- inflammatory, bacteriostatic, antimicrobial, deodorant, antiseptic, and spasmolytic activities, these activities may be due to cyanogenic glycosides and valerianic acid, It is have several compounds like Bitter prieiple, sugar, Triene, Quercetrine, Volitile oil, Resin, and Acid Tannic [19,20]. This work was amid to evaluation the effectiveness of *Syzygium aromaticum* and *Anthemis nobilis* alcoholic extracts against some isolates of pathogenic bacteria *Streptococcus pyogenes* that collected from patient with respiratory tract infections, and compare activity of them with inhibition activity of some antibiotics.

Materials and Methods

Collecting samples

Eight isolates of pathogenic bacteria *Streptococcus pyogenes* obtained from patients with respiratory tract infections using an anaerobic jar with a candle lit to continue growth because it prefers an oxygen-free atmosphere, The medium used is Blood agar.

Preparation of plant extract:

Material of plant were brought from local market, then washed with distilled water and 1% hypo chloride sodium solution, air dried and then ground into fine powder using mechanical grinder. For alcoholic extract 50 gr from the powdered of each plant dissolved in 500 ml (96%) ethanol and then placed in rotary shaker for 24 hours. After the end of 24 h, these solutions were filtered by using many sheets of muslin cloth, then the supernatant was collected and filtered with filter paper for many times to obtain a pure filtrate. Finally, the mixture was placed in the oven at 40 ºC to evaporate the solvent. Dried extract stored in the fridge for the later use. Five ml of distilled water was added to dissolve 0,5 gram of both extracts to prepare 100 mg/ml as a stock solution for the concentrations to be prepared (25, 50, 75) % mg/ml

Determination the Efficacy of Plant Extracts:

Eight identified isolates of *Streptococcus pyogenes* were diagnosis in the Department of microbiology at Tikrit University that isolated from patients with respiratory tract infections and cultured on the blood agar for 24 h at 37 ºC then stored in the refrigerator at 4 ºC.

The screening of plant extracts as antibacterial activity against *S. pyogenes* was carried out by disk agar diffusion method [21]. Bacterial suspension was prepared by transferring 3-5 pure bacterial colonies to glass tube containing physiological normal saline solution to compared the turbidity of bacterial suspension with the standard turbidity constant (McFarland tube) which gives an approximate cell count of $1.5* 10^8$ cell/ml depending on the guidelines of clinical and laboratory standard institute (CLSI). After that, by using sterile swab, to spread a portion of bacterial suspension on the surface of Muller- Hinton agar, wells have been done using cork borer and loaded with 50 µL of several dilutions of extracts, then the plates left to dry and placed in an incubator for 24 h at 37 ºC. Finally, diameter of inhibition zone was measured by a ruler [22].

Susceptibility Test Antibiotic:

Method of disk diffusion was used to test the sensitivity of bacterial isolates against the antibiotics which used: Meropenem (MEM), Amikacin (AK), Ciprofloxacin (CIP), Gentamicin (GN), Trimethoprim/ culphimethoxazole (SXT), Ceftriaxone (CRO), Piperacillin (PRL), Amoxicillin (AX) Table 1. These antibiotics were distributed on the surface of cutler media of bacterial suspension and incubated at 37 Cº for 24 h and the inhibition zone was recorded and compared with the standard rates of inhibition zone diameter for antibiotic that are internationally approved (CLSI) [23].

Table 1: Antibiotics used.

Antibiotic	Symbol	Con. Mg/disk	Sensitive mm	Resistant mm
Meropenem	MEM	10	≥ 37	≤ 29
Amikacin	AK	10	≥ 17	≤ 14
Cirpfloxacin	CIP	10	≥ 21	≤ 15
Gentamicin	GN	10	≥ 15	≤ 12
Trimethoprim/ sulphamethoxazole	SXT	25	≥ 16	≤ 10
Ceftriaxone	CRO	10	≥ 21	≤ 15
Piperacillin	PRL	100	≥ 22	≤ 14
Amoxicillin	AX	25	≥ 18	≤ 13

Nowadays, the resistance to drugs has increased because the random utilized of commercial drugs which are usually utilized to treat the symptoms of the diseases, creating it a universal rising problem. So, there is a crucial require to find novel antibacterial drugs from medicinal plants to treating the infectious diseases. Plants have long been considered a natural source of medicines. But, human inclines to disregard the significance of herbal medicine. Diverse extracts of folk medicinal plants were verified to recognize the source of its medicinal properties [24].

Results of the two extracts used against *S. pyogenes* as antibacterial were revealed that the alcoholic extracts of *Anthemis nobilis* did not show any antibacterial activity against all isolates of *S. pyogens*, while the alcoholic extract of *Syzygium aromaticum* showed a significant antimicrobial activity. The highest inhibition of experimental isolates of *S. pyogenes* was appeared at 100% and 75% concentration sequentially, while the isolates indicated a lower resistant to 50% and 25% concentration of cloves extract, the reason for this insensitivity may be due to the decrease in the concentration of active compound which increased with the increase concentration of extract. Results of antimicrobial investigation are presented in Table 2.

Table 2: Diameter of inhibition zone of alcoholic extract of *Syzygium aromaticum*

Bacterial isolates. No	Con 100% mm	Con 75% mm	Con 50% mm	Con 25% mm
1	36.5	30	0	0
2	36.5	36.5	33.5	35
3	37.5	0	0	0
4	38	33.5	0	0
5	32.5	37	0	0
6	0	0	0	0
7	37.5	35	29	0
8	32	37.5	32	29

The oil and buds of clove have antifungal, antioxidant, antiparasitic, antithrombic, and antimutagenic [25]. Humulenes, acetyl salicylate, eugenol, and acetyl eugenol, chavicol are the active ingredients in clove [26], in addition, clove contains several volatile substances such β-pinene, farnesol, 2-heptanone ethyl hexanoate, benzaldehyde, and limonene [27]. Based on the results of this study, it was found that alcoholic clove extract was more effective against most bacterial isolates at the concentration 100%. These active compounds, chiefly eugenol was damaged the membranes of bacteria and led to escape of intracellular materials [28]. Raja et al 2015 [29] reported that clove extract has the ability to reduce the formation of biofilms. Another study, El-Maati *et al*. (2016) [30] reported that phenolic clove extract has many properties such antioxidant and antimicrobial, and can be used in nutrition and pharmacological products as natural antioxidant and antimicrobial factor. Some studies pointed to the activity of *S. aromaticum* extract as antibacterial against various species of standard bacteria in addition to another isolated clinical species [14,31, 32].

Results of the sensitivity test of bacterial isolates to antibiotic showed that most of isolates were sensitive to all antibiotics used except CRO, as shown in Table 3 the diameter of inhibition zone. The results were discussed based to the guidelines of clinical and laboratory standard institute (CLSI guidelines, 2018) [23].

Table 3: Diameter of antibiotic inhibition zone

Antibiotic /mm **No. of Isolates**	**CIP**	**GN**	**CRO**	**PRL**	**SXT**	**AK**	**AX**	**MEM**
1	38	27	0	26	20	27	35	35
2	38	36.5	0	26	48	20	17.5	35.5
3	32.5	27	0	30	20	27	35	35
4	40	36.5	0	15.5	48	23.5	37.5	35.5
5	37	35	0	30	0	28	35	28
6	35	35	0	0	39.52	28	37.5	40.5
7	37	38	27	0	23.5	28	0	35.5
8	35	25.5	17.5	20	24.5	20	26	40

The ratio of bacterial sensitivity to used antibiotic was 100% to (CIP, AK, GN), 87.5% to SXT, and 75% to AX. While the ratio of bacterial resistant was 12.5% to CRO, 25% to MEM, and 50% to PRL, as shown in Table 4 and figure 1.

Table 4: Ratio of Antibiotics.

Antibiotic	CIP	GN	CRO	PRL	SXT	AK	AX	MEM
Ratio	100%	100%	12.5%	50%	87.5%	100%	75%	25%

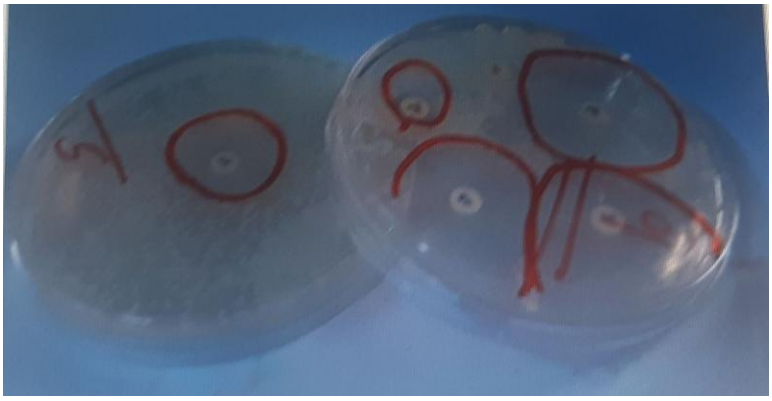

Figure 1: Inhibition activity of antibiotics.

Microbial resistance to antibiotics can be divided into natural resistance resulting from the functional structure of the bacterial cell and the nature of its genetic material, and acquired resistance resulting from the acquisition of new resistance mechanisms through acquired resistance genes from other bacteria [33]. In addition to the random and overuse of antibiotics which leads to mutations in bacteria and their resistance to antibiotics, as well as the horizontal transfer of resistant trait between different bacterial species. Bacterial resistance to antibiotic can also linked to the production of biofilms, which provides it the ability to survive and withstand difficult physical conditions, making treatment process very difficult because antibiotic target bacterial proteins that are produced only when bacteria are actively growing [34].

Streptococci can attachment with host cells and cause many diseases because they possess many surface receptors and many secretory proteins which they use for adhesion and colonization in different places in the host's body, thus leading to tissue destruction to enable them to spread.

Streptococcus pyogenes has numerous virulence factors that have an effect on its pathogenesis, for instance, the bacterial capsule consisting of hyaluronic acid, this capsule gives protection against phagocytosis. The cell wall of this bacterium is also described by its complex conformation, as it contains many antigens, the most important of which are peptioglycans, capsular polysaccharides, lipoteichoic acid, and several proteins such as F, R, T and M protein. In addition to producing many exotoxins and enzyme that simplify tissue invasion and contribute to tissue injury and inflammation allowing it to start infection and avoid the immune system including streptokinase, streptodornase, hyaluronidase, and streptolysins [35, 36]. Streptokinase has the ability to stimulate plasminogen and change it into active plasmin by binding to a protein alike to M protein, so it was considered as a spreading factor that simplifies bacterial invasion of tissue. Hyaluronidase is one of the enzymes that aids the degradation of hyaluronic acid in connective in host tissue, thus simplifying the spread of infection. Cystein protease SpeB is another important virulence factor has the ability to degrading several host factors like chemokine, immunoglobulin, plasminogen, and fibrinogen [37, 38].

The random and overuse of this antibiotic by the people without consulting a doctor, in addition to the longtime using of these antibiotics lead to developing the bacterial ability to resist many beta-lactam antibiotics. Also, streptococci produce penicillin- binding protein

(PBP) which located in the cytoplasmic membrane and it is attached to the cell wall. Theses protein is a target for both penicillin and cephalosporin antibiotics, as they change the target location of the beta-lactam antibiotics, which results in bacterial resistance to them [39]. And ability to produce the enzyme penicillinase, which breaks down the beta-lactam ring, in addition to interfering with the production of the peptidoglycan layer of the bacterial cell. The CIP antibiotic, which belongs to the Fluroquinolones family, works by affecting the genetic DNA inside the bacteria, which leads to the death of this bacteria. GN, AK antibodies belong to the Aminoglycoside family, which inhibits cell protein by affecting any of the bacterial ribosomes 30S and 50S. As for the PRL antibiotics, which belongs to penicillin family, it affects bacteria by binding to PBPs and inhibiting them, thus preventing the formation of peptidoglycan, although peptidoglycan is the most important component of most bacteria [40].

Conclusions

The ethanolic extract of *Syzygium aromaticum* (clove) showed the activity to exhibited preferential growth-inhibitory activity against *Streptococcus pyogenes* at concentration 100% and 75% showed an inhibitory capacity close to the inhibitory capacity of CIP, AK, GN.

The results showed that the inhibition zones were increased with the increasing of concentrations of extract thus it was very clearly that the results encourage the use of natural Sources like some plants or some parts of plants to solve some problems done by bacteria activities. Cloves extract can be used as an essential ingredient in various medicines. However, it requires further research and trials.

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تأثير بعض المستخلصات النباتية (القرنفل *Syzygium aromaticum* والبابونج *Anthemis nobilis*) ضد *Streptococcus pyogenes*

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الخلاصة:

ترتبط أمراض المكورات العقدية بعدوى الجهاز التنفسي والتي تعد سببا رئيسيا للأمراض والوفيات في جميع أنحاء العالم. أزداد الأهتمام بالمستخلصات النباتية والمركبات ذات الفعالية البيولوجية الأخرى والمعزولة من النباتات لعلاج الأمراض على نطاق واسع منذ القدم. يهدف هذا البحث الى تقييم الفعالية التثبيطية للمستخلصات الكحولية لنباتي القرنفل *Syzygium aromaticum* والبابونج *Anthemis nobilis* ضد ثماني عزلات من بكتريا من المرضى المصابين بألتهابات الجهاز التنفسي المراجعين لمستشفى تكريت التعليمي، ومقارنة هذه الفعالية الضد مايكروبية مع فعالية بعض المضادات الحيوية لتثبيط نمو البكتريا بأستخدام أختبار الحساسية للمضادات الحيوية. تم قياس الفعالية المضادة للميكوبات بأستخدام طريقة الأنتشار بالقرص. أظهرت النتائج أن جميع العزلات كانت أكثر حساسية للمستخاص الكحولي لنبات القرنفل عند التركيز 100% و أقل حساسية للتراكيز اللأخرى، في حين لم يكطن لمستخلص البابونج أي تأثير تثبيطي ضد جميع عزلات *S. pyogenes* أظهرت نتائج أختبار الحساسية للمضادات الحيوية أن جميع عزلات البكتريا كانت حساسة للمضادات CIP, GN, AK بنسبة 100% ، في جين كانت جميع العزلات مقاومة للمضادات الحيوية الأخرى بنسب مختلفة (CRO 87.59, AX 75%, PRL 50%, MEM 25%). تم الأستنتاج من هذه النتائج بأن الفعالية التثبيطية لمستخلص القرنفل الكحولي بنسبة 100%, 75% كان مقاربة لفعالية المضادات الحيوية CIP, GN, AKعلى نمو العزلات البكتيرية.