Study of the Synergistic Effect of Antimicrobial Drugs and Plant Extracts Against *Staphylococccus aureus* Strains

Maha A. Abidul –Ameer **Ghaida'a J. Mohammad**

Abstract

Antimicrobial drugs effective for treatment of patients infected with resistant *Staphylococcus aureus*(RSA) are limited .Thus ,it is important and valuable to find compound that potentiate antimicrobial activity of antibiotics on RSA. So, our study aims to evaluate the synergistic effect between two antibiotics (Ampicillin and Acaflex) and the extracts of *Eucalyptus* and *Thuja* plant against RSA.

We examined the anti-bacterial action of two plants(*Eucalyptus* and *Thuja*) extracts on plasma coagulation by *staphylococcus aureus* and the effect of conventional chemotherapy combined with plant extracts below the MIC. Coagulation was inhibited in plasma containing(10,20,50,70 and 100mg\L)for both extracts. The MICs of ampicillin and acaflex for *S.aureus* markedly reduced from(0.25,0.5 &1mg\L) of the four resistant strains to 0.06mg\L in muller hinton agar (MHA)plates with 20mg\L *Eucalyptus* extract and 50mg\L *Thuja* extract. The results suggest that *T. catappa* and *E. camaldulensis* extracts can be used in treating diseases caused by the test organisms. .

الخلاصة

إن العقاقير المضادة المؤثرة أو المفيدة لعلاج المرضى المصابين ببكتريا المكورات العنقودية المقاومة محدودة لهذا فمن الضروري وذا أهمية إيجاد مركبات تزيد من فعالية المضادات الحيوية للمكورات العنقودية المقاومة، لذلك فأن دراستنا تهدف إلى تحديد أمكانية التأثير التآزري للمضادين الحيوين (Ampicillin&Acaflex) مع مستخلص نبات الأيوكالبتوز و العفص ضد المكورات العنقودية المقاومة.

تم فحص الفعل المضاد لمستخلصين نباتين(الأيوكالبتوز والعفص) على تخثر البلازما من قبل المكورات العنقودية الذهبية والتأثير العلاجي للمستخلصات النباتية تحت التركيز المثبط الادنى.ثبط التخثر في البلازما الحاوية على (10،20،50، 70 و 100 ملغم/لتر)من كلا المستخلصين .أنخفض التركيز المثبط الأدنى لل- Ampicillinو ال-Acaflex لدرجة كبيرة لأربع عزلات مقاومة من المكورات العنقودية من(0.25،0.5 و 1ملغم/لتر).إلى 0.06 ملغم/لتر في أطباق MHA حاوية على (20 &50 ملغم/لتر) لمستخلص الأيوكالبتوز ومستخلص العفص على التوالي . توضح هذه النتائج أمكانية استخدام كلا المستخلصين في علاج الأمراض المتسببة من قبل المكورات العنقودية الذهبية المختبرة.

Introduction

Infectious diseases still represent an important cause of morbidity and mortality among humans, especially in developing countries. Even though pharmaceutical companies have produced a number of new antibacterial drugs in the last years, resistance to these drugs by bacteria has increased and it now become a global concern. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents (Nascimento *et al*.,2000).

Staphylococcus aureus is recognized as one of the major causes of infections in humans occurring in both the community and the hospital. Multidrug resistant staphylococci have become a major nosocomial pathogen(NNIS,2004).Therefore the importance of identifying new effective antimicrobial agents cannot be overemphasized. Among the potential sources of new agents, medicinal plants have long been investigated. In rational drug therapy, the concurrent administration of two or more drugs is often essential and sometimes mandatory in order to achieve the desired therapeutic goal or to treat co-existing diseases. However, the drug interaction may have different effects on the host as well as the infecting microorganism. The potential benefits of using combined antimicrobial therapy can be treatment of mixed infections, therapy of severe infections in which a specific causative organism is known, enhancement of antibacterial activity, reducing the time needed for long-term

antimicrobial therapy and prevention of the emergence of resistant microorganisms (Hugo and Russel, 1993 and Levinson and Jawetz,2002).

Drug synergism between known antimicrobial agents and bioactive plant extracts is a novel concept and has been recently reported by Nascimento *et al*.(2000) and Abu-Shanab *et al*. (2005).

Many studies reported the antibacterial activity of the *Eucalyptus* leaf extract (Babayi *et al*., 2004; Akin-Osanaiye *et al*.,2007 and Ayepola & Adeniyi 2008) and *Thuja* seed extract (Diğrak ,1996 and Diğrak *et al.*,2002) against many microorganism. Therefore, Our study aims to evaluate the synergistic effect between Ampicillin and Acaflex and the extracts of *Eucalyptus* and *Thuja* plant against RSA.

Methodology

Bacterial strains: Eighteen *Staphylococcus aureus* strains were isolated from patients'clinical specimens who attending the general Al-Qadisiah hospital. These isolates were examined for plasma coagulation and antimicrobial activities of Ampicillin and Acaflex, they were grown in tryptic soy broth at 37C̊ overnight incubation ,the bacterial cells were harvested by centrifugation at 6000 r.p.m. for about 10 min, then resuspended in a sterile normal saline solution and centrifuged again, the process was repeated three times and then the washing bacteria resuspended(Akiyama *et al*.,2001).

Plant extracts: The method of Okogun(2000) was used to obtain the plant extract, 50gm of *Eucalyptus* leafs and 50gm of *Thuja* fruits were washed by water and dried for about 48h at room temperature, then crushed and extracted 3x with 800 of 70% acetone(1h with continuous stirring).All extracts were rotary evaporated under vacuum to remove acetone, and stored into clean and dried airtight vials at room temperature to be ready for use.

Plasma coagulation:-Cell suspensions of *S.aureus*(1*10̊ cell per ml) were used for inoculation 0.5ml of rabbit plasma either alone(control) or supplemented with *Eucalyptus* extract(10,20,50,70 and 100mg\L) or *Thuja* extract (10,20,50,70and 100 mg\L),aclot (plasma coagulation) was looked after incubation for about 24 at 37C̊.

Determination of Minimum Inhibitory Concentration(MIC) of extracts and antibiotics: The MIC of *Eucalyptus* extract and *Thuja* extract against the four resistant strains of *S.aureus* was determined in Muller-Hinton agar(MHA) using the agar plate method and the MIC of Ampicillin and Acaflex was also examined in MHA and MHA with 20mg\L *Eucalyptus* extract and MHA with 50 mg\L *Thuja* extract using the method of Collins *et al*.(1995).

Results and Discussion

1-Plasma coagulation

A total of 18 *S.aureus* strains coagulate plasma alone after 24h incubation at 37C̊,while there were 4(22.22%),10(55.55%),14(77.77%) and 18(100%) negative strains to plasma coagulation in plasma containing *Eucalyptus* extract with concentration 10,20,50 and 70,100 mg\L respectively and 4(22.22%),8(44.44%),10(55.55%) and 18(100%) negative strains to plasma coagulation in plasma containing *Thuja* extract with concentration 20,50,70 and 100 mg\L respectively but there were no one reported in concentration 10 mg\L, these results are more clarified in (table 1).

Table (1): The percentage of *S.aureus* strains that have a negative result to plasma coagulation at different concentration of *Eucalyptus* and *Thuja* extracts.

Extracts	Extracts Concentration (mg\L)				
	10	**20**	**50**	**70**	**100**
Eucalyptus	22.22%	55.55%	77.77%	100%	100%
Thuja	0%	22.22%	44.44%	55.55%	100%

These findings indicate that there were only four resistant isolates of *S. aureus* reported with 10mg\L of *Eucalyptus* extract and 20mg\L of *Thuja* extract; thus it has been selected to evaluate the effect of both extracts on their growth and detect the synergistic effect between Ampicillin and Acaflex and the extracts of *Eucalyptus* and *Thuja* plant against RSA. .

2-MIC of Extracts

The data pertaining to the minimum inhibition concentration(MIC) of *Eucalyptus* extract and *Thuja* extract against the four resistant strain of *S. aureus* which are S3,S8,S14 and S17 presented in table (2) which explained that the MIC of *S. aureus* isolates was (20 & 50mg\L) for *Eucalyptus* extract catching two isolates for every concentration and (50 & 70mg\L) for *Thuja* extract, also two isolates were recorded for every concentration.

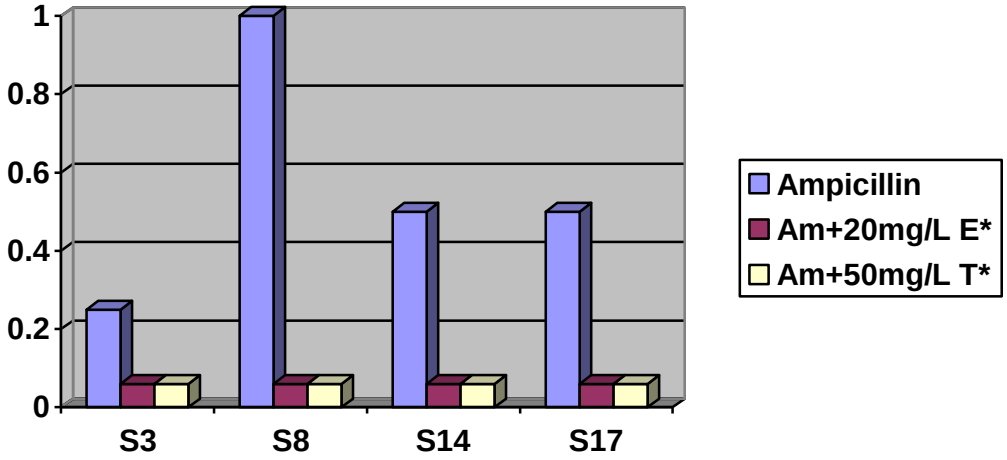
Table (2): The MICs(mg\L) of *Eucalyptus* extract and *Thuja* extract for *S.aureus* strains on MHA plates

Extracts	No. of strains with MIC			
	S3	**S8**	**S14**	**S17**
Eucalyptus extract	20mg/L	50mg/L	50mg/L	20mg/L
Thuja extract	50mg/L	70mg/L	70mg/L	50mg/L

Both *Eucalyptu*s and *Thuja* extract have an astringent effects refer to the present of tannins which known as an active antimicrobial agent against many microorganism (Odonovan and Brooker.,2001).These findings may be due to that source, concentration, and chemical properties are important factors that influence antimicrobial activity of tannin extracts(Min *et al*.,2008) where the antimicrobial mechanisms of tannins can be summarized as follows:(1)The astringent property of the tannin may induce complexation with enzymes or substrates. Many microbial enzymes in raw culture filtrates or in purified forms are inhibited when mixed with tannins.(2)A tannin's toxicity may be related to it’s action on the membranes of the microorganisms.(3)Complexation of metal ions by tannins may account for tannin toxicity(Akiyama *et al*.,2001).

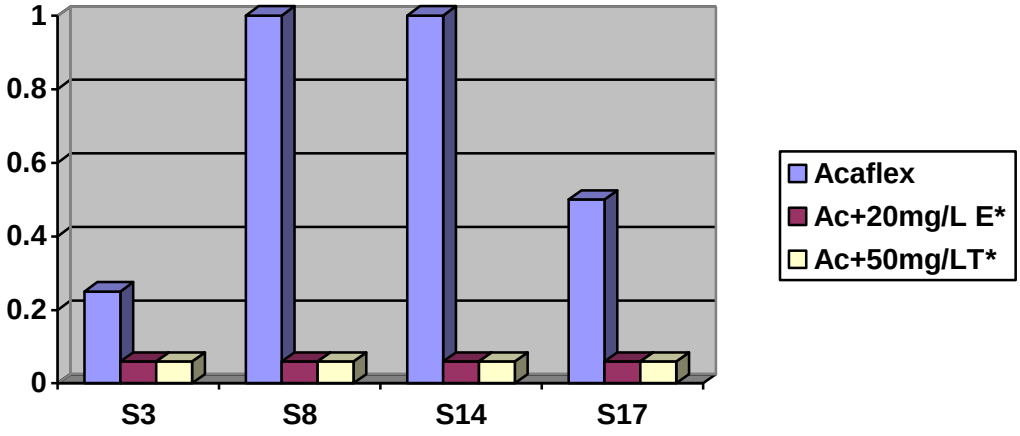
3-MIC of antibiotics and extracts

Figure(1) shows the MICs of Ampicillin for the four resistant strains of *S.aureus* in MHA with or without *Eucalyptus* extract (20mg\L) and *Thuja* extract (50 mg\L).As shown the MICs of Ampicillin decreased to 0.06mg\L in MHA with the 20mg\L *Eucalyptus* extract and 50mg\L *Thuja* extract.



Figure(1):The mininmal inhibition concentration(mg\L)of Ampicillin with and without *Eucalyptus* and *Thuja* extract for *S.aureus* strains on MHA plates. *Am=Ampicillin ,*E=*Eucalyptus*,*T=*Thuja*

Figure(2) shows the MICs of Acaflex for the four resistant strains of *S.aureus* in MHA with or without *Eucalyptus* extract (20mg\L) and *Thuja* extract (50 mg\L).The MICs of Acaflex also decreased to 0.06mg\L in MHA with the 20mg\L *Eucalyptus* extract and 50mg\L *Thuja* extract. .



Figure(2):The minimal inhibition concentration(mg\L) of Acaflex with and without *Eucalyptus* and *Thuja* extract for *S.aureus* strains on MHA plates. *Ac=Acaflex,*E=*Eucalyptus*,*T=*Thuja*

Since both Ampicillin and Acaflex posses antibacterial activity against RSA, it is necessary to assess whether the anti-RSA effect observed in the presence of the two antibacterial agents(two antibiotics and two plant extracts) is an additional one or a synergistic one. Therefore, the MICs of Ampicillin and Acaflex were determined against RSA strains in the absence or presence of (20mg\L) *Eucalyptus* extract and

(50 mg\L) *Thuja* extract respectively and it has been observed that the effect was a synergistic one. These findings may be due to the damage occurring in the cell wall and in the cell membrane caused by epigallocoatechin gallate and an increase in the permeability would be responsible for the potent synergy as reported by (Shimizu *et al*.,2001) .

The present study indicated that both extracts of the studied plants showed an increase in the antimicrobial activity of certain drugs that can be used against *S. aureus*, and synergistic interaction of plant extracts is possible with antimicrobial drugs and these results are consistent with previous reports which showed that some plant extracts can increase the activity of antimicrobial drugs in vitro against bacteria (Nascimento *et al.,* 2000 ; Junior *et al*.,2005; Betoni *et al* .,2006 and Chang *et al*.,2007) and also consistent with study of Shimizu *et al*.(2001) who found that an extract of *Arctostaphylos uva-ursi* markedly reduced the MICs of B-lactam antibiotics such as Oxacillin and Cetmetazole against methicillin resistant *S.aureus*(MRSA).This high synergism rate shows the need for more studies concerning the molecular basis of these interactions to understand the synergistic mechanism which is fundamental to development of pharmacological agents to treat bacterial infections using medicinal plants.

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