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Full Length Research Paper

Investigation of drug susceptibility in S. aureus isolated from burn patients in Missan city

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Twenty one isolates of *Staphylococcus aureus* collected from Alsader a hospital – Missan then were analyzed between (October and December in 2013). The samples taken in order to determine the bacterial profile and antibiotic susceptibility. Isolates of *S. aureus* were tested against 8 differrent antibiotics and crude of methanol extracts of *Capsicum annuum* L.var. (Solanaceae) by a disk diffusion method. 100% of the isolates were resistant to the Penicillin, 95% resistant to Lincomycin , 90.4% Tetracyclin. 90.4% Rifampin, 80.9% resistant to Nitrofuraantion and 57.1% resistant to Bacteriacin were found to be the least effective antibiotics while 9.5% resistant to Amicacine and 38.09% resistant to Novobiocine where more effective antibiotics against (MRSA) *S. aureus*. The minimum inhibitory concentration (MIC) (mg/ml) of the alcoholic were also determined. Also the antimicrobial suscepitlity of isolates where tested against methanol extract of *C. annuum* with different concentration (500, 250, 125, 62.5 µġ/ml) detected by agar well diffusion methods. The alcoholic extracts proved to be more effective than aqueoous extracts antimicrobials against (MRSA) *S. aureus*. Finally cytotoxicity evaluating towards human RBC, the results revealed these crud extracts of *C. annuum* have not any cytotoxicity in all coocentrations. The good antimicrobial potency of the methanol extract of *C. annuum* plants indicates the tretment of (MRSA) as an alternative to the costly antibiotics.

Key words: Antibiotic, minimum inhibitory concentration (MIC), MRSA (*Staphylococcus aureus*), *Capsicum annuum* L., cytotoxicity.

INTRODUCTION

Staphylococcus aureus is a cause of nosocomial infection and primary human pathogen, is widely distributed in nature, *S. aureus* is gram positive, coagulase positive, facultative anaerobic and microscopically characterized as a single , pars or closters cocci, non-motile, non – sporring, and catalase positive (Leticia *et a*l., 2013).
The resistant strain, MRSA which is widespread, particularly in the hospital sitting. *S. aureus* (MRSA) generally remained an uncommon finding even in hospital sittings until the 1990s when there was an explosion in (MRSA) prevalence in hospitals of *S. aureus* (MRSA) (Foster, 1996).

S. aureus become resistant to many commonly used antibiotics due to indiscriminate use of antibiotics. The β-lactamase – resistant penicillins (methicillin, oxacillin, cloxacillin and flocloacillin) were developed to treat penicillin –resistant *S. aureus.* Penicillinase – resistant penicillins are able to resist degradation by *Staphylococcal* penicillinase and are still used as first-line treatment. (John et al., 1996).In a survey 51% *S. aureus*

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Table 1. Eight types of antibiotics used in this study.

No	Antibiotic	Concentration	Company
1	Tetracycline	TE10 (mcg)	Bioanalyse
2	Penicilin	P10 (mcg)	
3	Amicacin	AK30 (mcg)	
4	Rifampin	RA 5 (mcg)	
5	Nitrofurantoin	F100 (mcg)	
6	Bactiracin	U10 (mcg)	
7	Lincomycin	L10 (mcg)	
8	Novobiocin	NV30 (mcg)	

Figure 1. *Capsicum annuum.*

were found to be MRSA, as well as the bacteria cause food poisoning, toxic shock syndrome, scalded skin syndrome (Medeiros, 2012).Antimicrobial resistance has become a global problem, The resistant strain MRSA which was first discovered in the UK in 1961.Now is widespread, particularly in hospital setting (Johnson *et al.*, 2004).

Burns are damage to the skin caused by a variety of non – mechanical sources including chemicals, electricity, heat, or nuclear radiation (Ouattara *et a*l., 1997). Burn wound infection is problematic because it delays healing, encourages scamming and may result in bacteremia , sepsis or multiple – organ dysfunction syndrome whereby organs from several systems are unable to maintain homeostasis on their own , requiring immediate medical attention (Church *et al*.,2006). Alternatively, Traditionally, *Capsicum annuum* L. var. longum (Solanaceae) which has a curly shape. Chillitinctur was previously reported to be able to inhibit the growth of *Staphyllococcus* sp*., Escherichia coli, Bacillus aureus and Bacillus subtilis1).* Is traditionally used to treat ox cuts before race 2. In Indonesia, chilli pepper is also traditionally used to treat oral thrush which is usually caused by *Candida albicans* (Sanucci *et al*., 2003).

This report presents was planning to investigate drug susceptibility in *S. aureus* isolated in one of Alsader a hospital – Missan city from burn patients , the activity of the methanol extracts of cultivars of Capsicum annuum, var. longum as Alternative traditional treatment.

MATERIALS AND METHODS

The study was done in the biology department of science college of missan university, from October and December in 2013 .

Sterilization: - All media autoclaved by autoclave for 20 min at 121 °c at pressure 1.5 PSI.

Plant material

Capsicum [Figure 1] was collected from the market in Amara city, and taken to the laboratory. The Plant materials (Fruit) were washed separately under running tap water, followed by rinse using sterilized distilled water. Excess of was removed from the plant material using filter paper before they were used for extraction.

Table 2. Susceptibilities of 21 *Staphylococcus aureus* 8 antibiotics.

Antibiotics	Resistant (no.)	Resistant (%)
1 Tetracycline	19	90.4
2 Penicillin	21	100
3 Bacteriacin	12	57.1
4 Rifampin	19	90.4
5 Nitrofurantion	17	80.9
6 Novobiocin	8	38.09
7 Amicacine	2	9.5
8 Lincomycin	20	95.2

Extract preparation

Aqueous extract

25 g of plant material was macerated with 400 ml distilled water at room temperature and then filtered using filter paper Wattman No.1 Filter paper under aseptic conditions and the filtrate was collected in sterilized glass Petri dish to dray in room temperature 24 h for evaluation of antibacterial activity.

Methanol extracts

25 g of plant material was macerated with 400 ml organic solvent (methanol). The mixture thus obtained was filtered through filtered using filter paper Wattman No.1 Filter paper. The filtrate was concentrated by evaporation of solvent at room at temperation. Extracts were stored at 4°C until further use.

Isolation and Identification of *Staphylococcus aureus*

A total of 21 bacterial strains including Gram-positive bacteria *S. aureus, S. epidermis*, were isolated from some burned patients, a sterile cotton swab was used. Samples were cultured on manitol salt agar medium incubated overnight at 37°C, the colony appeared then subcultured and purified. Identification of the isolates based on many characters such as colonial morphology, Gram stain, motility, catalase test and oxidative tests (Hananfy and Haten, 1991).

Antibiotic susceptibility assay

The well diffusion assay is suitable for aqueous extracts because they are difficult to dry on paper discs (Tadeg *et al*., 2005). A suspension of each isolate was made at turbidity equal to 0.5 McFarland standards and then plated on to Muller – Hinton agar Plate. Antibiotic disc was applied to each plate. The plates incubated at 37°C for 24 h. After incubation the inhibition zone was measured, the results of all isolates compared with standard isolates of *Staphylococcus aureus* were isolated from burned patients.

Method antimicrobial activity

Approximately 2-5 freshly grown bacterial colonies of the test organism were emulsified into Nutrient broth and incubated for 10-15 min at room temperature. With a spreader of wire loop, Mueller Hinton agar plate was evenly inoculated and plate allowed to stand for 5 min at room temperature (that is 250°C).Using a sterile agar borer (Sterilized using a Bunsen flame), wells were dug into the inoculated agar at reasonable distance apart (approximately 5 cm).

The plant extract(s) were transferred into the created agar wells till when full. Extract were not allowed to float on the agar surface. The plate lid was replaced and did not turn the petri-dish upside down. The setup was incubated at 37°C overnight. The presence for bacterial inhibition zones around each well looked for. Appearance of clear zones around well was indicative of the anti bacterial activity of an extracts (Bizimenyera et al., 2005).

Determination of MIC by agar plate dilution method

According to the methods of (NCCLS, 2002), agar plate dilution test was used to determine the Minimum Inhibitory Concentration (MIC) of an antimicrobial agent.

Determination of the minimum inhibitory concentration (MIC) of extract

Materials for Minimum Inhibitory Concentration (MIC) of extract Broth culture of test organism (s); known concentration of plant extracts (e.g. 0.5 g/ml, 1 g/ml etc); set of test tubes; micro-pipette (adjustable 100 - 1000 µl);

Table 3. Showed Antibiotic sensitivity test of pathogenic *Staphylococcus aureus* isolated from some patients in al Sader hospital in Amara city.

No	Antibiotic	Symbol	Bacterial strain/ *Staphylococcus aureus*
1	Tetracycline	TE	10mm
2	Penicilin	P	-
3	Amicacin	AK	28mm
4	Rifampin	RA	-
5	Nitrofurantoin	F	7
6	Bactiracin	U	12mm
7	Lincomycin	L	-
8	Novobiocin	NV	20mm

Disk diameter (6 mm).

Table 4. The mean of inhibition zone of aqueous and alcoholic of crude extracts from Capsicum fruits on the growth of MRSA.

MRSA.	The mean of inhibition zone of aqueous of crude extracts from Capsicum fruits on the growth of MRSA .			
	62.5 mg/ml	125 mg/ml	250 mg/ml	500mg/ml
S. aureus	0	0	0	0

Mean of three value of each concentration.

Table 5. The mean of inhibition zone of aqueous and alcoholic of crude extracts from Capsicum fruits on the growth of MRSA

MRSA.	The mean of inhibition zone of alcoholic of crude extracts from Capsicum fruits on the growth of MRSA.			
	62.5 mg/ml	125 mg/ml	250 mg/ml	500 mg/ml
S. aureus	0	7	16	21

*MRSA isolates bacteria. Mean of three value of each concentrate.

Nutrient broth; sterile physiological saline; incubator.

Method for minimum inhibitory concentration (MIC) of extract

A set of test tubes were dispensed 0.5 ml (500 µl) of physiological saline. An equal volume (that is 0.5 ml) of test plant extract were added to the saline in first tube and mixed the two thoroughly well (NCCLS, 2002).

Cytotoxicity assay

According to the methods of Xian - guo and Ursula (1994), human red blood cells were used for toxicity test.

RESULTS

Table 2 showed high resistance to Penicillin (100%), Lincomycin (95.2%), Tetracycline (90.4%), Rifampin (90.4), Nitrofurantion (80.9), Bacteriacin (57.1), Novobiocin (30.09), Amicacine (9.5). Multi-Deug resistant strain of Staphylococcus aureus were recorded from clinical (burn Patients).

In this study, agar well diffusion method was used to determine the antibacterial effect of the crude extracts of Capsicum fruits Figure 3.

The growth inhibition of bacteria increased as the concentration of extract increased, the susceptibility pattern to the extracts on *S. aureus* of alcoholic crude expressed maximum inhibitory zone at concentration 500 mg /ml which was 21 mm but in low concentration 125 mg /ml was 16.33 mm (Tables 3 to 6, Figures 2 to 4).

DISCUSSION

In our study showed high prevalence of bacterial infection especially *Staphylococcus aureus* among burn patiants. *S. aureus* remains a common colonizer and developed

Table 6. The MIC of the alcoholic extract of Capsicum annum fruit of *MRSA strains.

Sample	Dilution of alcoholic extract of *Capsicum annuum* (mg/ml)						
	≥64	≥32	≥16	≥8	≥4	≥2	≥1
**S. aureus*	-	-	-	-	+	+	+

* MRSA

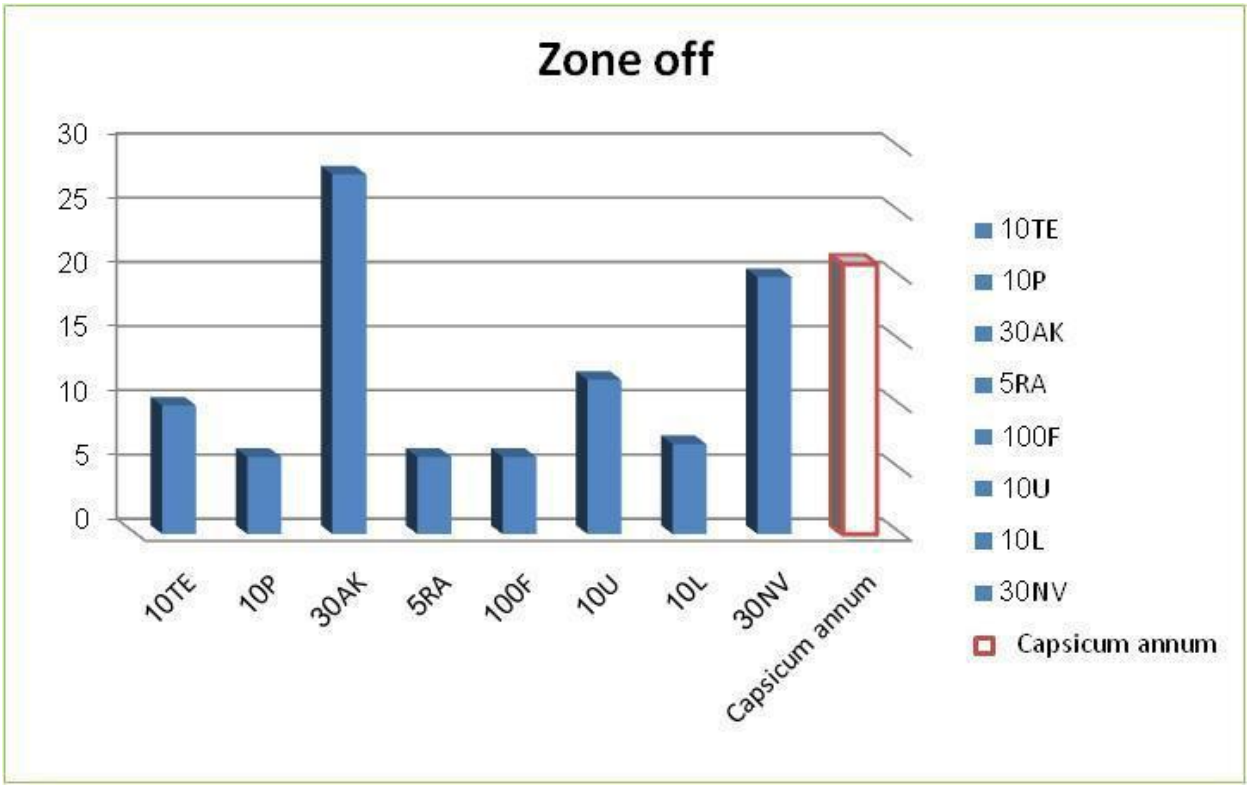

Figure 2. Effect of eight types of antibiotics and *Capsicum annum* methanol extracts on inhibition zone against pathogenic *Staphylococcus aureus* isolates bacteria.

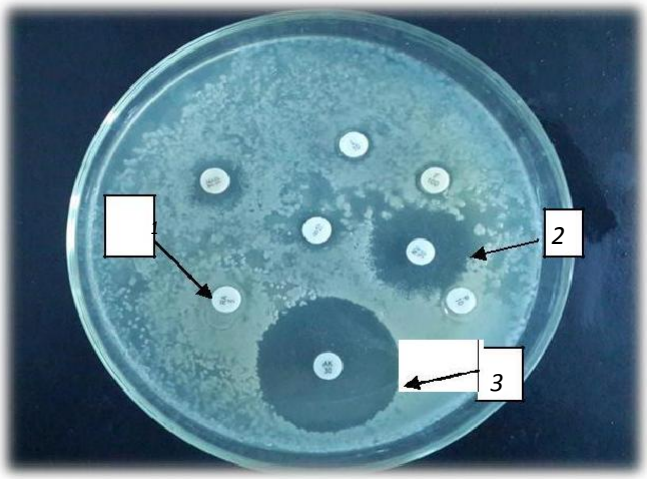

Figure 3. Mueller Hinton agar media with antibiotic sensitivity disc showing resistant and sensitive (arrow only 2, 3).

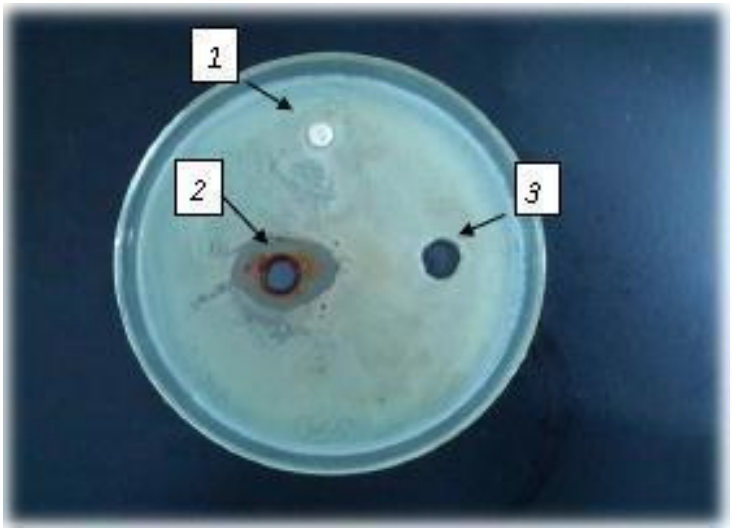

Figure 4. Mueller Hinton agar media with antibiotic sensitivity disc shows Penicillin resistant (arrow 1) and sensitive (arrow only 2 shown alcoholic extract of Capsicum annuum), (arrow 3) shown DMSO solvent.

resistance to several anti-microbial agents (Koffi-Nevry et al., 2012). The results prevalence of *Staphylococcus aureus* and new resistant was detected against Tetracycline 100%, Lincomycin 87%, Rifampin 84%, Nitrofurantion 93%, Penicillin 100%, Bacteriacin 91%, Amikacin was the most effective antibiotic followed by Novobiocin Table 2, Figure 3), this results are agreement with other studies (Russell, 2002). Multiple antimicrobial resistances of the bacterial pathogens are of great concern both in veterinary and human medicine worldwide. Antimicrobial resistance is a serious problem in the treatment of animal and human patients with infectious diseases. Like other bacterial pathogens *S. aureus* has become resistant to many antimicrobials through the acquisition of mobile drug resistance genes. A major force in the over expression of endogenous multidrug transporters and spread of plasmid encoded multidrug transporters in the huge consumption of antibiotics in human therapy, the pathogens inside hospital always in change due to exposure to different type of antibiotics and other antimicrobial medication such as disinfectant, detergins, antisepic etc)Zetola *et al*., 2005).

Multiple antibiotic resistances of bacterial pathogens are of great concern in human medicine worldwide, antibiotic resistance is a serious problem in the treatment of human patients with infection diseases (Safdar et al., 2006). Some of these colonization proteins also increase salt tolerance which could be beneficial in instances of infection where low perfusion results in high external osmolarity, such as in burn wounds, sometimes up to 20% greater than would be triggered by bacteria not in possession of this virulence factor, for these reasons that MRSA infections are so dangerous to patients whom are already immune-compromised (Syamsuhidayat et al., 1991).

The resistance of bacteria toward different drug can be due to modification of the target site, by pass of pathways, decreased uptake by reduced intracellular concentration of the antimicrobial agent, either reducing membrane permeability or by active efflux pump, enzymatic inactivation or modification of the drug, or overproduction of the target (Coates *et al*., 2002).

The antimicrobial activity of alcoholic extracts of *C. annum* fruit in Table 5, Figure 4 shows the effect of different concentration (62.5, 125, 250, and 500 mg /ml) of crude alcoholic extract which increased the inhibition zone against MRSA bacteria .The result agreement with (Hananfy and Haten, 1991).

Table 6 shows that The (MIC) value of the crude extracts of Capsicum fruits were (8 mg /ml) against gram positive MRSA bacteria. This may be explained by the fact that Gram positive bacteria, due to their structural features, are more susceptible to phenolic compounds than Gram negative bacteria (Ouattara et al., 1997). Also that agreement with study of Koffi-Nevry *et al*. (2012) showed the effect of *C. annuum* and Capsicum fratescens methanol and aqueous extracts on selected bacteria (*S. aureus*, *Salmonella typhimurium*, *Vibrio cholera, P. aeruginosa*, *E. coli* and *Shigella dysenteriae*) were investigated that result show both extracts were found to be effective against *V. choleriae* , *S. aureus* and *S. typhimurium*.

Finally a test was also carried out to examine the Cytotoxicity assay by using Xian-guo and Ursula (1994) methods towards human red blood cells in which the crude extracts of Capsicum fruits where found that they are not having cytotoxisity on (1-500 microgram/ml).

Results of this study suggest that the alcoholic extract of Capsicum annuum may be useful either alone or when combined with antimicrobial agents to treat MRSA bacterial infections.

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