CAPTER ONE

1. INTRODUCTION

1.1. Back ground

Bacterial infections are responsible for many deaths each year. Methicillin-resistant *Staphylococcus aureus* (MRSA) was mentioned in 1,230 death certificates. New antibacterial treatments are urgently needed **(Office for National Statistics News Release, 2009).**

Some herbs have been reported to have antibacterial activity. The compound thymol, extracted from thyme essential oil, was used as a battle field antiseptic in the First World War, whereas tea tree oil was used in the Second World War **(Castleman , 2001).**

Plant extracts have been widely used as topical and oral applications for diseases treatment. Examples of these include green tea, lemon, lavender, rosemary and papaya **(Moghbel *et al,* 2005).** Black tea is the second most commonly drank liquid on the earth after water. Green tea (*Camellia sinensis*) which is not fermented at all during drying process has numerous medicinal benefits mainly due to its antibacterial and antioxidant properties **(David and Sifton, 2004).**

Green tea is a popular drink, especially in Asian countries, although its popularity continues to spread across the globe. The health benefits of green tea, derived from the leaves of the *Camellia sinensis* plant, have been studied. Fairly recently, researchers have gone to look at the possibility of using green tea in antimicrobial therapy, and the potential prevention of infections. The particular properties of catechins found in the tea have shown promise for having antimicrobial effects. There are four main catechins (polyphenols) found in green tea: epicatechin (EC), epicatechin -3- gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG). Three of these, ECG, EGC, and EGCG have been shown to have antimicrobial effects against a variety of organisms. These catechins have exhibited a variety of antimicrobial mechanisms **(Wanda, 2014).**

Staphylococcus aureus (*S. aureus*) is a common commensal of humans and its infections have always been among the most common causes of morbidity and mortality **(Sheagren, 1984)**. Although primary *S. aureus* infections are not common, a great deal of the virulence from this organism occurs through cross infection by spread from patient to patient in hospitals and other institutional settings. In contrast, healthy individuals have a small risk of contracting an invasive infections caused by *S.aureus* but they can carry the organism **(Foster, 2004).** Because it's primary habitat is moist squamous epithelium of the anterior nares **(Aly and Levit, 1987).** Most invasive *S. aureus* infections are assumed to arise from nasal carriage (**Von Eiff *et al.*, 2001**). The severity of

infections caused by this bacterium rises in the presence of highly propensity for development of antibiotic resistance. Methicillin resistant isolates are of majorities.

1.2. Justification (Rationale)

Staphylococcus aureus infections and the emergence of MRSA strains in Sudan may constitute a public health problem with a strong potential for dissemination and high rates of morbidity and mortality. It causes infections ranging from wound infections to endocarditis. MRSA was infecting people who had chronic illness, but now it is becoming a serious problem within health care organizations and community individuals. Although conventional antibacterial agents are available such as penicillins, vancomycin, macrolides, cephalosporins and fusidic acid in considerable quantities, increase resistance to these drugs can result in treatment failure **(Foster, 2004)**.

Therefore, there is an important need to discovery of new, safer, cheaper and more effective antibacterial agents used by sudanese population.

Several studies have been done in Sudan for different plant extacts to render the importance of plants medicine such as **Farooq *et al* (2007)** on moringa oleifera and **Abd alfatah *et al* (2013)** on four plants species found in west of Sudan.

Therefore, it is of interest to carry such study to provide individuals with new plant types to be used as safer and claimed antibacterial agent and with better care in case of MRSA infection or colonization. .

1.3. Objectives

1.3.1. General objectives

To study antibacterial activity of green tea against *Staphylococcus aureus* nasal carriage among health care workers in Omdurman Teaching Hospital.

1.3.2 Specific objectives

1- To determine the frequencies of *S. aureus* nasal carriage in health care workers.

2-To determine the frequencies of MRSA nasal carriage in health care workers.

3- To detect the antibacterial activity of green tea of methanol and aqueous extracts against *Staphylococcus aureus* nasal carriage.

4- To determine the minimum inhibitory concentration (MIC) of methanol and aqueous green tea extracts against *Staphylococcus aureus* nasal carriage.

5- To identify the major chemical compounds of green tea as analyzed by gas chromatography.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Nature and pharmacological properties of green tea

2.1.1. Origins and nature of tea

Tea was originated in China, possibly as long ago as 2700 BC. Drinking water, boiled for reasons of hygiene, was made more palatable by the addition of leaves from the tea plant **(Peter *et al.*, 2005).** The word 'tea' has been used to describe the shrub *Camellia sinensis,* family *Theaceae* **(Hamilton-Miller, 1995).**

2.1.2. Chemistry of green tea

The chemical composition of green tea is complex and is incompletely defined. The most abundant components in green tea are polyphenols, in particular flavonoids such as the

catechins, catechin gallates and proanthocyanidins. The fresh leaves contain caffeine

(approximately 3.5% of the total dry weight, or about 50 mg/cup when brewed),

theobromine (0.15–0.2%), theophylline (0.02–0.04%) and other methylxanthines, lignin

(6.5%), organic acids (1.5%), chlorophyll (0.5%) and free amino acids (1–5.5%), in addition to the unique amino acid theanine (4%); numerous 'flavour compounds' are also present in much lower amounts **(Graham, 1992).** Many of the biological properties of green tea have been ascribed to the catechin fraction, which constitutes up to 30% of the dry leaf weight. These potent antioxidants comprise free catechins such as catechin, gallocatechin, epicatechin (EC) and epigallocatechin (EGC), and the galloyl catechins such as epicatechin gallate (ECg), epigallocatechin gallate (EGCg), catechin gallate (Cg) and gallocatechin gallate (GCg). EGCg is the most abundant of these, comprising about 50% of the catechin pool; EGC accounts for around 20%, ECg 13% and EC 6% **(Hara, 2001)**.

2.1.3. In vitro antimicrobial activity of green tea extracts

In study done by **Doa'a and Rowida, (2014)** showed effects of water extracts green tea using disc diffusion method against Gram positive bacteria like MRSA (methicillin resistant *Staphylococcus aureus*) were isolated from hospitalized patients' different sources (pus and wound) and Gram negative bacteria including *E. coli* and *P. aeruginosa*. Green tea was showed antimicrobial activity against MRSA with inhibition zone 19.67 ± 0.33mm and MIC 1.25 ± 0.00mg/ml compared with standard reference antibiotics (vancomcin) 18.00 ± 0.00mg/ml.

Other study conducted by **Maksum *et al* (2013)** concluded that *Camellia sinensis* leaves water extract could be useful in combating emerging drug resistance caused by MRSA and *P. aeuroginosa* using disc diffusion and MIC methods. The results showed that the inhibition zone diameter of green tea extracts for *S. aureus* ATCC 25923 and MRSA were (18.970) mm and (19.130) mm respectively. The MIC of green tea extracts against *S. aureus* ATCC 25923 and MRSA were 400 μg/ml and 400 μg/ml, respectively, whereas the MIC for *P. aeruginosa* ATCC 27853 and MDR- *P. aeruginosa* were 800 μg/ml, and 800 μg/ml, respectively.

Also, **Jiehyun *et al* (2014)** conducted a study to investigate the minimum inhibitory concentrations (MICs) of EGCG and GTE in *Pseudomonas aeruginosa* and *Escherichia coli*, and assess the use of these chemicals as an alternative or adjunct topical antimicrobial agent against *P. aeruginosa* and E. *coli* with multidrug resistance. The *P. aeruginosa*

and *E. coli* strains used in this study showed multidrug resistance. EGCG inhibited the growth of *P. aeruginosa* at a MIC level of 200-400 μg/ml. The MIC of GTE was a 1: 16 dilution for *P. aeruginosa.* EGCG showed antimicrobial activity against *E. coli* at a MIC of 400 μg/ml. In the case of green tea water extract, the MIC was at dilution between 1 : 8 and 1 : 4 for *E. coli*, which confirms that EGCG and GTE showed potential as alternative

or adjunct topical antimicrobial agents for infections that are resistant to traditional antibiotic therapy.

According to **Abdolmehdi *et al* (2013)** who investigated the activity of green tea extract on some clinically isolated cariogenic and periodontopathic bacteria. Green tea extract was prepared by aqueous extraction method and diluted from 50 to 1.56 mg/ml. Standard techniques of agar disk diffusion and broth microdilution assays were applied for qualitative and quantitative determinations of antibacterial activity of green tea extract on each isolates. The result of this study gives rise to that all clinical isolates of *S. mutans* (100%) were sensitive to green tea extract at concentrations 6.25, 12.5, 25, and 50 mg/ml producing inhibition zones ranging from 10 to 38 mm. All periodontopathic isolates (*A. actinomycetemcomitans*, n = 20, *P. intermedia*, n = 20, and *P. gingivalis*, n = 20) (100%) tested were sensitive to 12.5, 25, and 50 mg/ml of this extract. The MIC of green tea extract for *S. mutans* was 3.28 ± 0.7 mg/ml and for *A. actinomycetemcomitans* 6.25, for *P. gingivalis* and *P. intermedia*12.5 mg/ml, which also confirm that green tea extract exhibited strong antibacterial activity on *S. mutans, A. actinomycetemcomitans, P. gingivalis* and *P. intermedia* and therefore may be used in mouthwashes or dentifrices for prevention of dental caries and periodontal diseases.

Another study showed that green tea leaves extracts were tested for antibacterial activity

against various bacteria isolated from environmental sources. All the extracts were tested for antibacterial activity by disc diffusion method. Antibacterial assay was performed at different concentrations. Significant antibacterial activity was reported for all extracts with results. Aqueous extracts has shown little antibacterial activity against six bacteria isolated. Maximum antibacterial activity was found in methanolic extracts. This study reflects the chemotherapeutic use of green tea **(Amit *et al.*, 2012).**

Activity against various other microbial pathogens or factors involved in their virulence has been shown; these include viruses such as hepatitis, HIV **(Fassina *et al.*, 2002),** rotavirus, enterovirus and influenza virus **(Song *et al.*, 2005),** yeasts **(Hirasawa and Takada 2004),** filamentous fungi **(Okubo *et al.*, 1991),** *Chlamydia, Mycoplasmas* **(Abdolmehdi *et al.*, 2013)** and parasites **(Paveto *et al.*, 2004).**

2.2.Genus *Staphylococcus*

2.2.1. Definition, pathogenesis and pathogenicity of *S. aureus*

Staphylococcus aureus is a species of the genus *Staphylococcus*, which contains mostly Gram positive within the family *Staphylococcaceae. S. aureus are* spherical bacteria of about 1 micrometer in diameter that occur in clusters under light microscope. Also, they have characteristic grapes like appearance seen under light microscope **(Wilkinson, 1997)**

S. aureus is one of the commonest causes of opportunistic infections in which are acquired in both community and hospital. It causes a variety of suppurative (pus-forming) infections and also

superficial skin lesions such as boils, styes and furuncules. In addition, it causes more serious infections such as pneumonia, mastitis, meningitis, urinary tract infections and food poisoning. Deep-seated infections such as osteomyelitis and endocarditis are also included **(Tenover *et al.*, 2000)**

There are different ways in which *S. aureus* gains access to the host. The most common way is the direct skin to skin contact. Some of which remain localized at the site of entry by the normal host defenses such as hair follicles. While the other gain access through needle stick or a surgical wound. Another way of entry is the respiratory tract causing staphylococcal pneumonia and it is a frequent complication of influenza. However, a number of virulence factors have been expressed by *S. aureus* to interfere with the host defense mechanisms. Some of them are capsular polysaccharides and protein A. These are surface factors that have been implemented to inhibit phagocytosis **(Foster and Höök, 1998)**. In addition to that, a number of enzymes such as leukocidin, coagulase, staphylokinase, lipase and hyaluronidase have been contributed to facilitate the spread and invasiveness within the tissues. Coagulase enzyme also has a role to interfere with the phagocytosis. Toxins that produced by strains of *S. aureus* such as enterotoxins, toxic shock syndrome toxin and exofoliative toxin also have been contributed to the invasiveness and pathogenicity **(Bohach and Foster, 1999)**

2.2.2. *Staphylococcus aureus* nasal carriage

Humans are natural reservoir of *Staphylococcus aureus* and asymptomatic colonization is far more common than infection. *Staphylococcus aureus* nasal carriage, affecting about 20% of the population. It has been identified as a risk factor for the pathogenesis of community acquired and nosocomial infections **(von Eiff *et al.*, 2001).**

2.2.3. Methicillin Resistant *Staphylococcus aureus* (MRSA)

MRSA is a bacterium responsible for difficulty to treat infections in humans. It may also be referred to as multi drug resistant or oxacillin resistant *Staphylococcus aureus* (ORSA). MRSA is by definition a strain of *Staphylococcus aureus* that is resistant to a large group of antibiotics called beta lactam, which include penicillins and cephalosporines. *S. aureus* is sometimes termed a superbug because of its ability to become resistant to several antibiotics **(Bilal and Gedebou, 2000).**

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study design

Descriptive – cross sectional study.

3.2. Study area

Omdurman Teaching hospital.

3.3. Study population

People included in this study were staff workers of Omdurman Teaching Hospital.

3.3.1. Inclusion criteria

Health care workers who were apparently healthy individuals.

3.3.2. Exclusion criteria

Community members (non health care workers).

3.4. Sampling

Non –probability sampling.

3.4.1. Sample size

Samples supposed to be collected according to the formula (n= 126)

but they were not all accessible. So, 100 swabs were obtained from the health-care workers.

$n= (z)^2 p q / d^2$.

n= The desired sample size.

z= The standard normal deviation (1.96).

p= The proportion in the population target estimated to have particular characteristics (0.5).

q= 1.0 – p (0.5)

d= Degree of accuracy (0.05).

3.5. Study variables

Screen on *Staphylococcus aureus* nasal carriage (dependent variable).Age and gender taken by members as (independent variables).

3.6. Data collection

The data were collected by using a direct interviewing questionnaire.

3.7. Ethical clearance

Permission of this study was obtained from the local authorities in the area of study, the objectives of the study clearly and simply were explained to all individuals participating in the study, verbal informed consent was obtained. **(Appendix V)**

3.8. Data analysis

All collected data were analyzed using Microsoft Office Excel 2007 and SPSS (Statistical Package of Social Science) soft program version 11.5.3.9 experimental work. Pearson Chi-square test was used for testing different significance. P value ≤ 0.05 was considered significant.

3.9.1. Sampling method

Nasal swabs were taken from healthy individuals using sterile cotton wool swabs. These swabs were rotated four times inside each anterior nares of each member. Each specimen was labeled with the same serial number given to the person whom undergoing swabbing. Then specimens were immediately transported to the laboratory and processed within two hours.

3.9.2. Culture

The Manitol salt agar (Oxiod) plate was divided into four quarters. Each sample was inoculated. Onto single quarter, streaking was done for each sample by platinum wire loop. The plates were inoculated aerobically over night at 37°C.

3.9.3. Colonial morphology

Yellow fermented colonies were selected to be identified by conventional methods.

3.10. Identification technique

3.10.1. Gram stain

Gram staining technique was used to help identifying pathogens in specimens and cultures by their reaction (either Gram positive or Gram negative) and morphology.

Staining was performed by preparing smear on clean slide; several colonies were emulsified in normal saline, the smear was allowed to air-dry. Then the smear was fixed by passing the slide rapidly three times through the flame. Then the smear was covered with crystal violet stain for 30 seconds. After that the stain was washed off with clean tap water followed by covering the smear with lugol's iodine for 30 seconds. Then the iodine was washed off with clean water. After that, it was decolorized rapidly for few seconds with acetone-alcohol then washed immediately with water. The smear was covered with neutral red stain for two minutes. At last the stain was washed off with clean water. The smear was dried and examined under oil immersion by light microscope **(Chessbrough, 2000).**

3.10.2. Catalase test

The catalase test is important in distinguishing streptococci (catalase-negative) from staphylococci which they are catalase positive. Catalase act as a catalyst in the breakdown of the hydrogen peroxide to oxygen and water. The organism was tested for catalase production by bringing it into contact with hydrogen peroxide.

The test was performed by pouring 2- 3 ml of 3% hydrogen peroxide solution into a test tube. By using sterile wooden stick several colonies of the test organism were removed and immersed in the hydrogen peroxide solution. Catalase-positive cultures bubbled at once **(Chessbrough, 2000).**

3.10.3. Coagulase test

Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of *S. aureus* and they are; free coagulase which converts fibrinogen to fibrin by activating a coagulase- reacting factor present in plasma and detected by clotting in the tube test. The other type is bound coagulase (clumping factor) which converts fibrinogen direct to fibrin without requiring coagulase reacting factor. It can be detected by the clumping of bacterial cells in the rapid slide test **(Chessbrough, 2000).** .

To prepare suspension for coagulase test, few drops of distilled water were added on the slide and then a various colonies (2 - 4) of the fermented organism were emulsifed in that drop to make thick suspension. After that a loop full of the EDTA anticoagulant human plasma was added plasma using sterile wire loop. Then mixing was done. These suspensions examined for clumping of the organism that occur within ten seconds **(Chessbrough, 2000).** .

3.10.4. DNase test

This test is used to identify *S. aureus* which produce deoxyribonuclease (DNase) enzymes. DNase test is particularly useful when the results of coagulase test are difficult to interpret. The test organism has been cultured on a medium which contains DNA. The test and control strains were inoculated as spot in media contains DNA by using a sterile loop or swab. After overnight incubation, the colonies were tested for DNase production by flooding the plate with 1mol/l hydrochloric acid solution. The excess acid was tipped off. The acid precipitates unhydrolyzed DNA. DNase- producing colonies are surrounding by clear area due to DNA hydrolysis and that was occurred within 5 minutes **(Chessbrough, 2000).**

3.11. Storage

In order to do subsequent sensitivity tests in one patch, preserving *S. aureus* isolates is recommended. From coagulase positive isolates *S. aureus*, several colonies were inoculated in Brain-heart infusion-glycerol broth by using sterile platinum wire loop. Then incubated in the cryoprotective tubes aerobically over night at 37°C. After that, incubated tubes were kept to the -20 °C refrigerator.

3.12. Green tea extraction

Extraction was carried out according to method described by **Sukhdev *et al.*, (2008)**

3.12.1. Preparation of the methanol extract

Fifty grams of green tea powder was extracted with methanol using soxhelt extractor apparatus. Extraction was carried out for about eight hours till the colour of solvent at the last siphoning time returned colorless. Solvent was evaporated under reduced pressure using rotary evaporator apparatus. Finally, extract was allowed to air in Petri dish till complete dryness and the yield percentage was calculated as followed:

Weight of extract obtained / Weight of green tea x100

3.12.2. Preparation of the aqueous (water) extract

Fifty grams of green tea sample was soaked in 500ml of hot distilled water, and left till cooled down with continues steering. Extract was filtered using filter paper and lyophilized using freeze dryer apparatus till complete dryness and the yield percent was calculated as pervious.

3.13. Antimicrobial susceptibility test

S. aureus isolates were sub cultured onto nutrient agar (Oxoide) from Brain-heart infusion-glycerol broth to obtained fresh isolated colonies. The antibiotics used in this study were penicillin and cefoxitin in the form of discs, 10 units and 30 micro grams respectively.

In this study this test was performed by standardized disc diffusion methods (Kirby – Bauer) **(Bauer *et al.*, 1966)**

3.13.1. Inoculation

Two to three colonies of the tested organism were picked with a wire loop from the culture plate and inoculated into test tubes containing 2 ml of peptone water. These tubes were then incubated for 3 to 5 minutes to produce a bacterial suspension of moderate cloudiness. The suspensions were compared with the 0.5 McFarland standard(0.5 =10^8 cfu/ ml) **(appendix IV).** After that, suspensions were inoculated onto Muller Hinton agar plates. The excess fluid was removed by pressing the swab against the side of the tube before plates were seeded. Then, the bacterial broth suspension was spread evenly in 3 directions on the surface of Muller Hinton Agar (Oxoide) with a cotton swab. Plates were Rotated approximately 60 degree to insure even distribution.

3.13.2. Applying of antibiotic disc

After the inoculums had been dried (3 to 5 min), the penicillin and cefoxitin discs were placed on the agar with flamed forceps and gently pressed down to ensure contact. Plates were incubated immediately at 37°C aerobically for 16-18 hours.

3.13.3. Preparation of green tea extracts discs

Whatman filter paper No.1 was cut into 6 millimeter discs and sterilized by hot air oven at 160 °C.for two hours.

3.13.4. determination of minimum inhibitory concentration (MIC) by disc diffusion method

0.4 grams of each extract was dissolved in 4ml of distilled water for water extract and 100% methanol for alcohol extract in separate tubes, then two fold serially diluted to obtain final concentration (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml). Ten micro liters of each prepared concentrations were then added to into the corresponding filter paper. The plates were then left for half an hour, and then incubated at 37°C for 24 hours. Inhibition zone around each disc were then measured using a ruler in millimeters. MIC is the lowest concentration of plant extract that did not permit any visible growth of the inoculated test organism.

3.13.5. Interpretation of Results

After overnight incubation, the zone diameters were measured with a ruler on the button of the Petri dish, the end point was taken as complete inhibition of growth as determined by the naked eye. These measurements classified the isolates into susceptible, intermediate and resistant categories according to zone diameter interpretive standards provided by the National Committee Clinical Laboratory Standards (NCCLS) Antimicrobial susceptibility test. About the green tea discs, equal or more than 9 millimeters interpreted as (sensitive), less than that interpreted as (resistant).

3.14. Quality control procedure

3.14.1. Control of culture media

The performance of manitol salt agar and nutrient agar culture media were controlled by testing each batch with known strain of *S. aureus*, and then checked after overnight incubation for expected characters of growth.

3.14.2. Control of susceptibility testing method

3.14.3. Reference strain quality control

The quality control *S. aureus* strain ATCC 25923, was used in the standard disk diffusion susceptibility testing methods was obtained from microbiology lab, Sudan University. This

reference strain was recommended for controlling the susceptibility tests as described in NCCLS document M7 –A7. The stock culture was stored at -20°C in 10% glycerol broth and sub cultured on to agar plate to obtain freshly isolated colonies. Control strain was suspended for testing according to the recommended inoculums preparation procedures.

3.14.4. Batch quality control

Each new batch of disc diffusion plates was tested with the reference strain to determine if zone diameter obtained with the batch fall within the expected range or not. Also one uninoculated agar plate from each batch was incubated overnight to ensure the medium's sterility.

3.15. Phytochemical screening

The general phytochemical screening for the active constituents was carried out for the most effective methanol extract of green tea using the Gas Chromatographic Mass Spectroscopy (GC-MS). In gas chromatography, the mobile phase (or moving phase) was Hellium. The stationary phase was a microscopic layer of liquid or polymer on an insert solid support inside a piece of glass or metal tubing called a column (a homage to the fractionating column used in distillation).

The instrument used to perform gas chromatography is called Chromatograph (or Aerograph, gas separator). The gaseous compound is being analyzed interact with the walls of the column, which is coated with y phase a stationary phase. This causes each compound to elute at a different time, known as retention time (RT) of the compound. The comparison of retention times were what gives GC its analytical usefulness **(Suleiman, 2013)**

CHAPTER FOUR

4- RESULT

Among the 100 nasal swabs included in this study, 19 (19%) *S. aureus* strains were isolated (Figure 1, Table 1). These isolates were primarily isolated on manitol salt agar, identified by Gram stain and other biochemical tests, as catalase and coagulase tests.

4.1. Culture

Some of the specimens isolated showed yellow fermented colonies on manitol salt agar plates while others showed pink non-fermented colonies due to presence of phenol red indicator (Figure 2).

4.2. Gram stain

Gram stain was done for isolates that ferment manitol and all of which showed Gram positive cocci in clusters. Such organisms appeared dark purple in color.

4.3. Identification

From yellow- fermented colonies, all 19 isolates collected were catalase positive. These isolates were confirmed with coagulase test, which all of them showed positive coagulase reaction. These isolates were clearly differentiated from coagulase negative isolates with no clumping reaction appear within 10 seconds.

4.4. Antibacterial susceptibility test

The susceptibilities of *S. aureus* isolates to penicillin and cefoxitin were determined using standardized disk diffusion method (Figure 3). The results showed that all *S. aureus* isolates 19

were resistant to penicillin. Fifteen (79%) were susceptible to cefoxitin while the reminder 4(21%) were cefoxitin resistant. Cefoxitin resistant isolates termed to be MRSA. (Figure3, 4, Table 2)

Table 1: Number of *S. aureus* carrier and non carrier health workers

Total sample number	***S. aureus* carrier**	***S. aureus* non carrier**
100	19 (19%)	81 (81%)

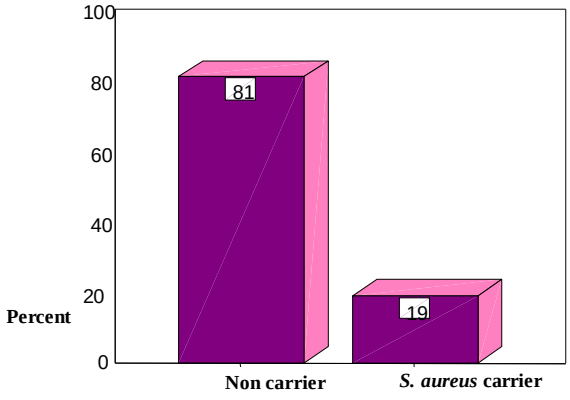

Figure 1: Percentage of *S. aureus* nasal swabs carriage isolated from health care workers.

Table2: Susceptibility of *S. aureus* isolates and *S. aureus* ATCC 25923 strain to penicillin and cefoxitin

Bacteria species	**Total No.**	**No. sensitive to pencillin**	**No. resistant to pennicillin**	**No. sensitive to cefoxitin**	**No.resistant to cefoxitin**
***S. aureus* isolates**	19	_	19	4 (21%)	15 (79%)
***S. aureus* ATCC** 25923	1	_	1	_	1

Keys zone of inhibition in millimeter**s**

Penicillin**;** Resistant < 28

Sensitive > 29

Cefoxitin; Resistant < 21

Sensitive > 22

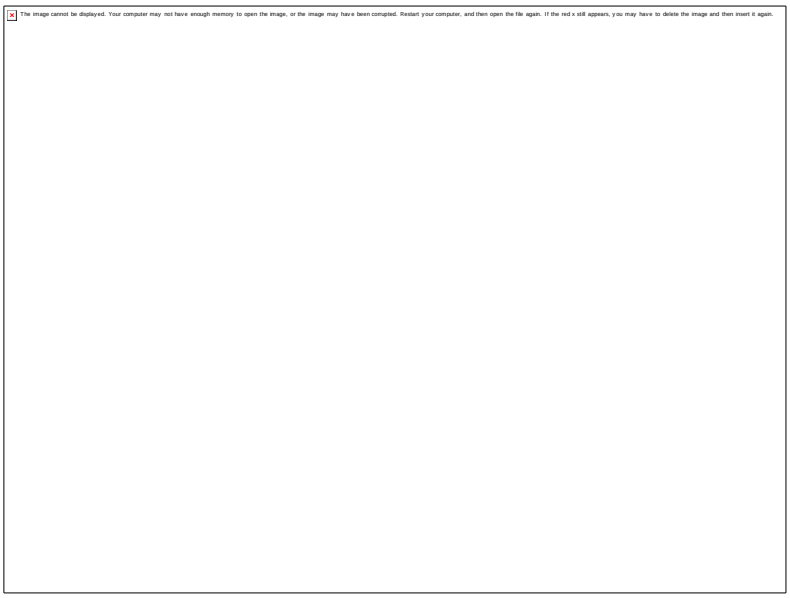

Figure 2: Growth onto manitol salt agar plate showing yellow fermentation of *S. aureus* and pink non fermented colonies.

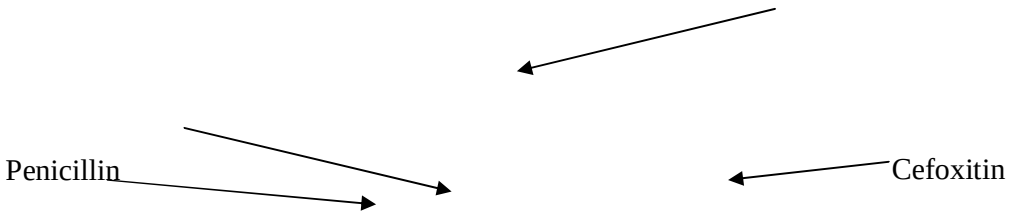

Figure 3: Antimicrobial susceptibility testing of *S. aureus* isolates to penicillin and cefoxitin using standardized disk diffusion method.

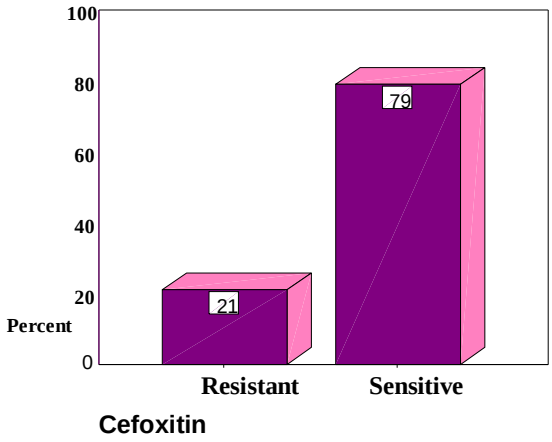

Figure 4: susceptibility of *S. aureus* to cefoxitin among health care workers.

4.5. Antibacterial activity of Green tea

Table3: Weigh and yield %s of extracts obtained by different solvents

	Aqueous		Methanol	
	Weigh of extract	**Yield %**	**Weigh of extract**	**Yield %**
Weigh of Green tea				
50 grams	11.6 grams	23.2 %	18.3 grams	36.6 %

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In this study, both water and methanol extracts of green tea showed antibacterial activity against strains of *S. aureus* nasal carriage isolated from health care workers, MRSA and *S. auerus* ATCC *(P=0.001*,Table 4, 5).

Table 4: The inhibition zones of different concentrations of water extract of green tea on bacterial isolates. Diameter of inhibition zone in millimeters.

Bacterial species	**100 mg/ml**	**50 mg/ml**	**25 mg/ml**	**12.5 mg/ml**	**6.25 mg/ml**
***S. aureus* ATCC** 25923	16	12	8	resistant	resistant

***S. aureus* nasal carrriage**	12-16	9-11	7-8	resistant	resistant
Methicillin – resistant *S. aureus* (MRSA)	9-10	7-8	resistant	resistant	resistant

Table 5: The inhibition zones of different concentrations of methanol extract of green tea on bacterial isolates. Diameter of inhibition zone in millimeters.

Bacterial species	**100 mg/ml**	**50 mg/ml**	**25 mg/ml**	**12.5 mg/ml**	**6.25 mg/ml**
***S. aureus* ATCC** 25923	18	14	11	8	resistant
***S. aureus* nasal carrriage**	15-21	11-18	9-11	7-8	resistant
Methicillin – resistant *S. aureus* (MRSA)	12-18	10-16	7-9	resistant	resistant

4.6. Minimum inhibitory concentration (MIC) of Green tea obtained by agar diffusion method

The MIC of Green tea water and methanol extracts obtained by agar diffusion method are shown in table 6 and figure 5 and 6.

Table 6: Minimum inhibitory concentration (MIC) of water and methanol Green tea obtained by agar diffusion method

Bacterial species	Water extract	Methanol extract
***S. aureus* ATCC** 25923	25 mg/ml	12.5 mg/ml
***S. aureus* nasal carrriage**	25 mg/ml	12.5 mg/ml
Methicillin – resistant *S. aureus* (MRSA)	50 mg/ml	25 mg/ml

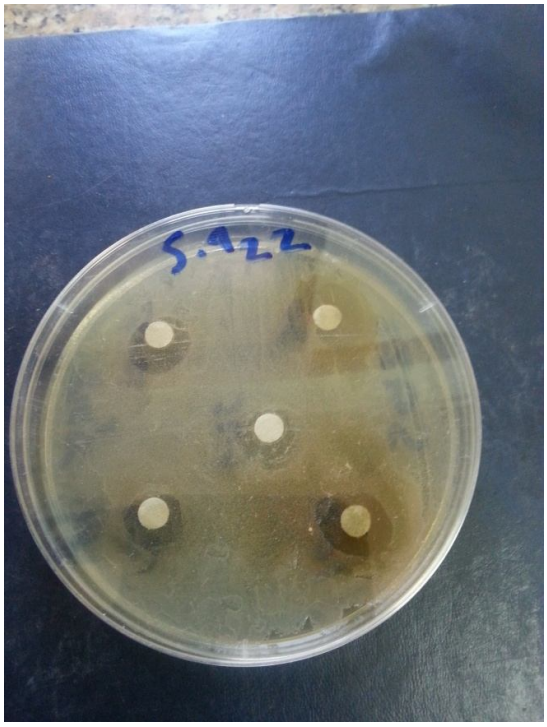

Figure 5: Activity of green tea methanol extract of different concentrations against *S. aureus* isolate

4.7. Gas chromatography results

Table 7 and figure 6 revealed that green tea (methanol extract) contain 23 phytochemical compounds. The active ingredients were not identified in this study.

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Table 7: Gas chromatography analysis of green tea

Peak	R. Time	Area	Area %	Name
1	5.637	31769	0.12	1,2,5,6- Tetrahydropyridin-2- one methyl
2	7.035	19638	0.08	3,4-Dimethyle- 3- pyrrolin-2- one
3	8.326	40745	0.16	Hydroquinone
4	8.24	39997	0.16	Benzofuran, 2,3-dihydro-
5	10.951	114433	0.45	2- Hydromethyl-2-methyl-pyrrolidine
6	11.089	291512	1.13	1,2,3-Benzenetriol
7	14.064	19554	0.08	1- Pentadecene
8	15.759	91369	0.36	1,2,3,5-Cyclohexanetetrol
9	16.058	4801406	18.68	Imidazo[4,5-e]- dione
10	17.147	70175	0.27	Purine-2,6(1H,3H)- dione
11	17.318	13228421	51.45	Caffeine
12	17.629	220335	0.86	Theobromine
13	18.716	82546	0.32	Hexadecanoic acid,methyl ester
14	18.839	512984	2.00	Squalene
15	19.063	92296	0.36	Levomenthol

16	19.003	68083	0.26	Methyl 10- trans-12-ci5
17	19.118	38213	0.15	9,12,15- Octadecatrienoic acid
18	19.200	113699	0.44	Phytol
19	19.263	53967	0.21	9,12- Otadeatrienoic acid(Z,Z)
20	19.301	38420	0.15	Ethyl Oleate
21	20.311	272340	1.06	Hexadeanoic acid, 1-(hydroxymethyl)
22	21.145	3719194	14.47	Z,E-2,13- Octadecaien-1-ol
23	21.518	1748693	6.80	9- Octadecenoi acid, 1,2,3-propanetriyl
		25709789	100.00	

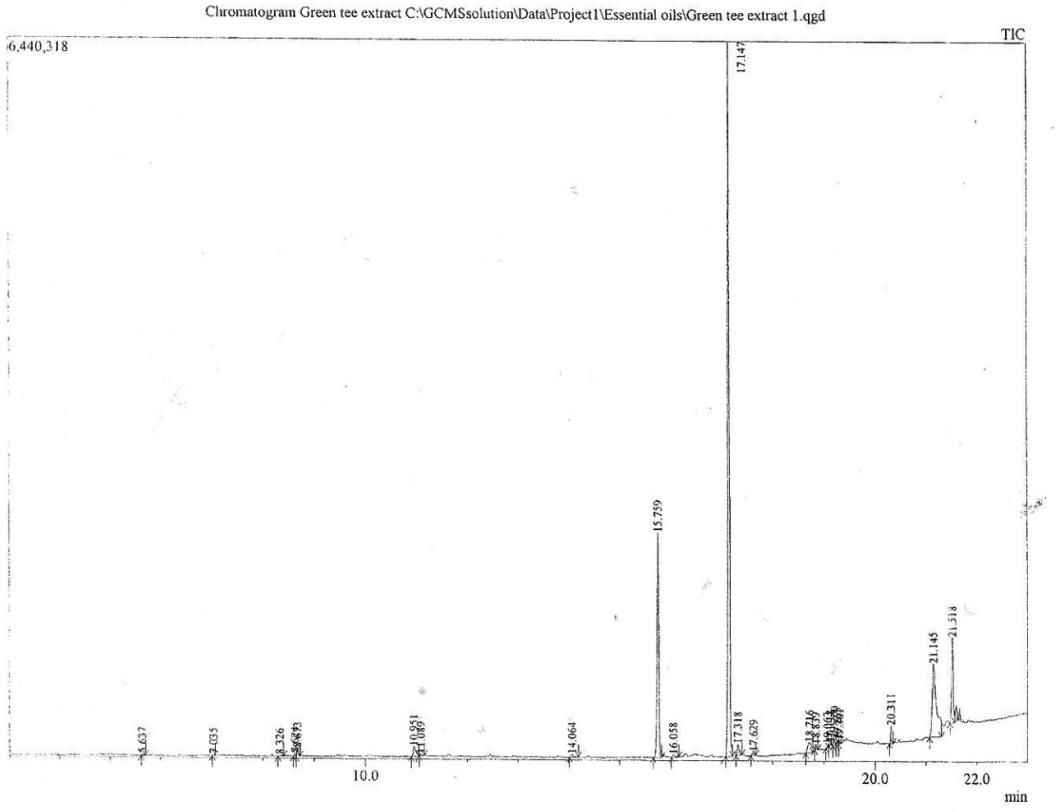

Figure 6: The phytochemical compounds found in green tea

CHAPTER FIVE

5. DISCUSSION

5.1. Discussion

The presence of *S. aureus* nasal colonization can provide an indication of a higher risk for subsequent infection, including MRSA (**Kluytmans *et al*., 1997)**. Therefore, the increased resistance of *S. aureus* to antimicrobials is a cause for concern.

In the present study, water and methanol extracts of green tea showed antibacterial activity against all the isolates of *S. aureus* nasal carriage from health care workers, MRSA and *S. auerus* ATCC 25923. This is in agreement with study conducted by **Doa'a and Rowida (2014)** who showed the effects of water extracts of green tea using disc diffusion method against MRSA (methicillin resistant *Staphylococcus aureus*) isolated from hospitalized patients, but with differences in the diameter zone of inhibition and MIC. In this study the inhibition zone of MRSA range between 9-10 and the MIC was 50 mg/ml whereas in **Doa'a and Rowida (2014)** the zone of inhibition was 19.67 ± 0.33 mm and MIC was 1.25 ± 0.00mg/ml.

In other study done by **Maksum *et al* (2013)** it is concluded that *Camellia sinensis* leaves water extract could be useful in combating emerging drug resistance caused by MRSA and *P. aeuroginosa* using disc diffusion and MIC methods. Their results showed that the inhibition zone diameter of green tea extracts for *S. aureus* ATCC 25923 and MRSA were (18.970) mm and (19.130) mm respectively. The MIC of green tea extracts against *S. aureus* ATCC 25923 and MRSA were 400 µg/ml and 400 µg/ml, respectively. This result is in disagreement with our study in MIC range and with slightly higher zone of inhibition.

Others studies conducted by **Jiehyun *et al* (2014)** and **Abdolmehdi *et al* (2013)** showed that green tea has antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli*, cariogenic , periodontopathic bacteria, *Mycoplasma* and *Chlymedia* respectively, which partially in agreement with our study that green tea has antibacterial activity.

In our study, it was found different results in both MIC and zone of inhibitions regarding green tea antibacterial activity between water and methanol extracts (table4, 5 and 6).

It was found that methanol extract of green tea is effective in inhibiting bacterial growth more than water extract and this is may be due to that green tea active ingredients have propensity to dissolve in methanol more than water.

The result of gas chromatography exhibited 23 compounds of green tea and also these compounds were identified qualitatively by the retention time, and quantitatively by the area under the curve. The active compounds which inhibited the growth of bacteria may be one or more of these 23 compounds, so further study must be performed to identify the compounds and the active ingredients. Some of these compounds have antibacterial activity against *S. aureus* .

5.2. Conclusion

1. *S. aureus* nasal carriage rate among healthcare workers in Omdurman Teaching Hospital which was found to be 19 %.

2. The prevalence of MRSA among healthcare workers was found to be 21%.

3. Green tea extracts (both water and methanol) shows antibacterial activity against *Staphylococcus aureus* nasal carriage isolated from healthcare workers.

4. Methanol extract of green tea is effective in inhibiting bacterial growth more than water extract.

5. MIC of methanol and aqueous green tea extracts was 12.5 mg/ml and 25 mg/ml respectively against *Staphylococcus aureus* nasal carriage isolated from health care workers.

5.3. Recommendations

1. It is recommended to isolate and identify the active ingredients in the compounds extracts responsible for the antibacterial activity.

2. Determination of minimum inhibitory concentrations (MICs) on different bacterial strains.

3. More research is required to verify these results.

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Appendices

Appendix I: equipments

Sensitive balance	1
Autoclave	1
Microscope	1
Incubator	1

Appendix II: Glass ware and consumables

Petri dishes	500
Slides	1 box
Test tubes	100
Wire loops	2
Sterile cotton swab	500

Kryo-vial	150

Appendix III: Culture media

All culture media were obtained from Oxoid.

(1) Manitol salt agar oxoid code CM0085:

Formula:	g/l
Peptone	10
Lab-lemco powder	1
Manitol	10
Sodium chloride	75
Phenol red	0.25
Agar	15

PH 7.5±.2

Directions:

Suspend 111g in 1 liter of distilled water, bring to the boiler to dissolve completely then sterilize by autoclaving at 121 °C for 15 minutes. When the medium has cooled to 50-55 °C, mix well and dispense it in sterile petri dishes.

(2) Nutrient agar medium oxoid code CM7

Formula	g/l
Yeast extract	1.0
Lab lemco powder	2.0
Peptone	5.0
Sodium chloride	5.0

Agar 15.0

PH 7.4 ± 0.2

Directions:

Suspend 28g in 1 liter of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes.

1. Nutrient broth oxoid code CM1

Formula: g/l

Lab lemco powder 1.5

Yeast extract 3.0

Peptone 5.0

Sodium chloride 5.0

PH 7.4 ± 0.2

(3) Peptone water oxoid code CM9

Formula: g/l

Peptone 10.0

Sodium chloride 5.0

PH 7.4 ± 0.2

Directions:

Add 15g to 1 liter of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121°C for 15 minutes.

(4) Muller Hinton agar oxoid code CM337:

Formula: g/l

Beef dehydrated infusion	300.0
cansein hydro lysate	17.5
Starch	1.5
Agar	1.7

PH 7.4 ± 0.2

Modified to meat performance standards.

This lots meet the NCCLS standard M6-A for dehydrated Muller Hinton agar.

Directions:

Add 38g in 1 liter of distilled water. Bring to the boil to dissolve medium ingredients completely. Sterilize by autoclaving at 121°C for 15 minutes.

(5) Brain-heart infusion-glycerol broth LAB MTM

Formula:	g/l
Brain-heart infusion solids	17.5
Tryptose	10
Glucose	2.0
Sodium chloride	5.0
Disodium hydrogen phosphate	2.5

PH 7.4 ±0.2

Directions:

Weight 37g powder disperse into 1 liter of deionized water, in addition to that add 40 ml of purified glycerol, allow to soak for 10 minutes then gently heat to dissolve before dispensing into tubes and sterilized at 121°C for 15 minutes.

Appendix IV: Reagents

Preparation of 0.5 McFarland standard

This is a barium sulphate standard against which the turbidity of the test and control inocula must be compared. Prepare 1%v/v solution of sulphuric acid by adding 1ml of concentrated sulphuric acid to 99 ml of distilled water , on the other hand prepare 1% w/v solution of barium chloride by dissolving 0.5 g of dehydrate barium chloride in 50 ml of distilled water. Mix 0.5 ml of barium chloride solution to 95.5 ml of the sulphuric acid, and mix. Transfer a small volume of the turbid solution to a screw cap bottle of the same type as used for preparing the test and control inocula. These tubes should be tightly sealed and in a dark place at room temperature (20-28 °C)

Appendix V:

Antibacterial Activity of Green Tea against *Staphylococcus aureus* Nasal Carriage Among Health Care Workers in Khartoum State

Submitted by: Moram Mohammed AbdulRahim Yousif

اقرار بالموافقه على المشاركه في البحث

أنا.....

بعد ان تم لي شرح طبيعة الدراسة واعطيت فرصة كافية للاستفسار عنها وقد تم الاجابه على كل اسئلتي بصورة كافية وفهمت ان لي الحق في التوقف عن هذه الدراسة في اي وقت و اوافق طوعيا على المشاركة في هذة الدراسة.

اسم المشارك في البحث..... التوقيع..... التاريخ.....

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