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Quality of guava leaves and tow herbals tea and their antimicrobial effect on *Staphylococcus aureus*

جودة أوراق الجوافة ونوعان من الشاي العشبي وأثرها المضاد على البكتيريا العنقودية

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Dedication

To our families

To the great motherland (Sudan)

To all our teachers and friends with great regard and respect.

Acknowledgements

Unlimited thanks to **ALLAH** who helped and gave us health to complete this work.

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We thank our brothers and friends who help us to do this work.

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Abstract

The aim of this study was to evaluate the quality of three type of plant , *Psidium guajava, Matricaria chamomilla L. and Rosmarinous officinallis* leaves (in form of tea),samples of tea were coxed (A-F) were A = 100% Guava, B= 100% Rosemary, C= 100% Chamomile, D= (homogenized sample 50%Guava\25% of two other herbs), E= (homogenized sample 50%Rosemary\25% of two other herbs) F= (homogenized sample 50%Chamomile\25% of two other herbs)the samples where soaked in the water and heated until the juice extracted then filtered and after that amount of sugar approximately (20%) was added, and subjected to the panelist for physiochemical characteristics, and to evaluate the effect of three type of herbs mentioned above on the growth of the pathogenic bacterium *Staphylococcus aureus* by use cub diffusion method. It is concluded that these three type of herb teas can be consumed as tea of acceptable characteristics and healthy at the same time, the fifty percent extract of *Psidium guajava,* was preferred over the other once. It was found the three type of tea had an antimicrobial effect on *S. aureus*, however *Psidium guajava* leaves tea was most effective on the growth of *S. aureus* compared to the other two types.

ملخص الدراسة

هدفت هذه الدراسة لإجراء التقييم الحسي لثلاثة أنواع من الشاي من مستخلص أوراق الجوافة والبابونج وإكليل الجبل حيث تم تقسيم العينات إلى ستة مجموعات (أ-و) بنسب مختلفة كالأتي: أ= 100% مستخلص جوافة، ب= 100% مستخلص الإكليل، ج= 100% مستخلص البابونج، د= 50% مستخلص جوافة و25% مستخلص الإكليل ومثلها من البابونج، ه= 50% مستخلص الإكليل و25% من مستخلص الجوافة ومثلها من البابونج، و= 50% بابونج و 25% مستخلص ورق الجوافة ومثلها من الإكليل، حيث تم نقع أوراق النباتات الجافة وغليها في الماء إلى أن أستخلص العصير ومن ثم تم تصفية المستخلص وتقديمه كشاي للمتذوقين بعد إضافة السكر (20%). وكذلك لقياس التأثير المايكروبايولوجى للثلاث أنواع على الباكتيرية العنقودية الممرضة بإستخدام طريقة الإنتشار. وخلصت الدراسة إلى إمكانية إستهلاك الثلاثة أنواع من المستخلصات كشاي ذو صفات حسية مقبولة وصحية في نفس الوقت، مع أفضلية مستخلص الجوافة. بالإضافة لذلك أظهرت الدراسة تأثير مضاد للنمو المايكروبي للثلاثة أنواع من المستخلصات العشبية عند إختبار تأثيرها على نمو البكتيريا العنقودية الممرضة، مع تفوق مستخلص الجوافة.

Chapter One

1.Introduction

Folk medicine is habitually used by low income people such as farmers, people of small isolate villages and native communities for the treatment of common infections. In these types of medicine, different types of plants which are prepared in many forms; for examples ingested as decoctions, teas and juice preparations are used. They can be also prepared into a poultice and applied directly on the infected wounds or burns **(Rojas *et al.*, 2006)**. Plants are known to produce variable compounds to protect themselves against a variety of pathogens, it is expected that plant extracts showing target sites other than those used by antibiotics which will be active against drug-resistant pathogens **(Sen and Batra, 2012)**. The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies **(El Astal *et al.*, 2005)**.

Staphylococcus aureus or "*staph*" is a type of bacteria found on human skin, in the nose, armpit, groin, and other areas. While these germs don't always cause harm, they can make you sick under the right circumstances. *S. aureus* is the leading cause of skin and soft tissue infections, such as abscesses, boils, furuncles, and cellulitis (red, swollen, painful, warm skin). S. aureus germs can also cause more serious infections, such as pneumonia, bloodstream infections, endocarditis (infection of the inner lining of the heart chambers and heart valves), and bone and joint infections. *S. aureus* is spread by touching infected blood or body fluids, most often by contaminated hands **(Chang and Chang, 2006)**. *Staphylococcal* food poisoning (SFP) is one of the most common food-borne diseases in the world following the ingestion of *Staphylococcal enterotoxins* (SEs) that are produced by

entero toxigenic strains of *coagulase-positive staphylococci* (CPS), mainly *Staphylococcus aureus* and very occasionally by other *Staphylococci* species such as *Staphylococcus intermedius* **(Hennekinne et al., 2012)**. Guava leaves have high antibacterial activity in extracts that can inhibit the growth of *S. aureus* **(Naseer *et al.*, 2018*)***. In many studies staph. Growth Was found to be inhibited invtro by many herbs **(Nieto *et al.*, 2018, Naseer *et al.*, 2018, Londonkar and Ranirukmini, 2010, El Astal *et al.*, 2005, Sen and Batra, 2012)**.

One of these herbs is *Rosemarinus officainalis L.*, popularly known as rosemary. It is used as a natural preservative in the food industry , widely used as a spice in cooking and in traditional medicine as medicinal plant to prevent and cure colds, rheumatism, pain of muscles and joints **(De Oliveira *et al.*, 2019)**.

Other well-known herb is Chamomile ***(Matricaria chamomilla** L.)*, popularly known as babong, is one of the important medicinal herb native to southern and eastern Europe. It is also grown in Germany, Hungary, France, Russia, Yugoslavia, and Brazil. It is a herbal plant that has been used for centuries in many human cultures to treat various inflammatory conditions such as eczema, ulcers, gout, neuralgia and rheumatic pains **(Singh *et al.*, 2011)**.

The third natural plant which may be used in ayurevidic therapy and found to inhibit staph. Growth is *Psidium guajava L.*, popularly known as guava, is a small tree belonging to the myrtle family *(Myrtaceae*). Native to tropical areas from southern Mexico to northern South America, guava trees have been grown by many other countries having tropical and subtropical climates. Traditionally, preparations of the leaves have been used in folk medicine in several countries, mainly as anti-diarrheal remedy. **(Díaz-de-Cerio *et al.*, 2017).**

Consumers are concerned about the negative effect of synthetic chemicals in food, so there is a need to find "clean label products". Therefore, there is a growing interest in using natural extracts as alternatives for synthetic additives because of their synergy with other preservation methods they are considered safe, and their specific properties as antioxidant, antidiabetic, antimutagenic, antitoxigenic and antibacterial. In general, herbs and plants are rich in compounds with antioxidant properties, such as vitamins (E and C), glutathione, enzymes and phenolic compounds. Several spice extracts have shown their properties to prevent the autoxidation of unsaturated triacylglycerol's **(Nieto *et al.*, 2018).**

Nowadays, due to increasing of resistant oboist antibiotic and their negatives sides we need to go back to our traditional herbs and organic once. Scientific research has proven there are many types of herb used n folk medicine, and prove its capabilities to inhibit microorganisms and its extracts treat diseases. In Sudan there are many medicinal plants while need to be studied.

Objectives

General objective

To evaluate the quality of three type of herbs as tea and their antimicrobial effect on gram-positive *Staphylococcus aureus*.

Specific objectives

1. To extract juice from the three herbs (*Psidium guajava L., Rosemarinus officainalis L.* and *Matricaria chamomilla L.)* and made into tea.

2. To determine sensory properties of different herbal tea prepared.

3. To study effect of herb mentioned above *on Staphylococcus aureus* Gram-positive.

Chapter Two

2. Literature Review

The successive extract of *Butea frondosa* have been investigated for antimicrobial activity against *Staphylococcus aureus* , *Pseudomonas aeruginosa, Klebsiella pneumoniae*, *E.coli* and fungi like *Aspergillus niger*, *Aspergillus flavus*, highest zone of inhibition was shown by LONDONKAR *et al*.**(Londonkar and Ranirukmini, 2010).** Sage, thyme and parsley are widespread medicinal plants in Palestine and widely used in folkloric medicine in treating different disease symptoms. Aqueous extract phenolic compound extracts from the leaf of these plants in addition to their commercial oils were investigated for their antimicrobial activity against ten pathogenic microorganisms. The "hole plate" diffusion method was used in testing various concentrations of these extracts. The results show had inhibitory effects against most tested microorganisms **(El Astal *et al.*, 2005)**. All the extracts from ten medicinal plants used in Colombian folkloric medicine showed varying degrees of antimicrobial activity on the microorganisms tested.**(Rojas *et al.*, 2006)**.

2.1. Psidium guajava

Which is considered a tree of great benefit (stem, leaf's, fruit and roots) **(Díaz-de-Cerio *et al.*, 2017).**

2.1.1. Use in traditional medicine

More recent studies show that *Psidium guajava* is used in many parts of the world for the treatment of a number of diseases, e.g. as an anti-inflammatory, etc… Some of the countries with a long history of traditional medicinal use of guava (leaf and fruit) include Mexico USA and other in Asia and Africa **(Gutiérrez *et al.*, 2008)**.

2.1.2. Leaves

Guava leaf's extract is being used as a medicine in cough, diarrhea, and oral ulcers and in some swollen gums wound. Guava leaves contain many compounds which act as fungistatic and bacteriostatic agents. Essential oil is present in leaves which contain α-pinene, limonene, β-pinene, and caryophyllene. Oleanolic acid is also found in the guava leaves. Leaves of guava have a lot of volatile compounds. Terpinene are present into the aqueous extract of plant's leaves which shows antimicrobial activity. Quercetin, and morin can be isolated from leaves. These compounds show the anti-oxidant activity **(Naseer *et al.*, 2018)**.

2.1.3. Antimicrobial

The inhibitory effects of aqueous and alcoholic extracts of the *Psidium guajava* (root as well as leaves) on the growth *of Staphylococcus aureus, Streptococcus mutans, pseudomonas aeruginosa, Salmonella enteritidis, Bacillus cereus, Proteus spp., Shigella spp.* and *Escherichia coli* , causal agent of intestinal infections in humans were examined using the in vitro agar well diffusion method **(Gutiérrez *et al.*, 2008).** Some studies were evaluating the antimicrobial effects of guava tree leaf extracts on diarrhea-causing bacteria. Carried out on *Staphylococcus aureus, Salmonella spp.* and *Escherichia coli* strains isolated from sea bob shrimp as well as on laboratory culture strains.**(Gonçalves *et al.*, 2008)**

2.1.4. Anti-diarrheal

Diarrhea has long been recognized as one of the most important health problems faced globally particularly by the population of developing countries. Ethanol and aqueous extracts of *Psidium guajava* exhibited can inhibit diarrhea. The antidiarrheal action of the extract may be due, in part, to the inhibition of the increased watery secretions that occur commonly in all acute diarrheal diseases and cholera **(Gutiérrez *et al.*, 2008)**.

2.1.5. Antimalarial

Guava leaves and stem extract was examining with sensitive strain of malarial parasite. In another study, leaves and stem bark of *Psidium guajava* inhibited *Entamoeba histolytica* growth (**Gutiérrez *et al.*, 2008)**.

2.1.6. Antioxidant

Dried leaves of *Psidium guajava* were extracted with hot water. The total phenolic content in extract was determine spectra photometrical according to Folin–Ciocalteu's. A remarkably high total phenolic content was obtained. Antioxidant properties are associated with its phenolic compounds **(Gutiérrez *et al.*, 2008)**.

2.1.7. Anti-inflammatory

Psidium guajava leaves is used worldwide for the treatment of various inflammatory ailments including rheumatism. The numerous polyphenolic compounds, triterpenoids and other chemical compounds present in the plant may account for the observed anti-inflammatory and analgesic effects of the leaf extracts **(Gutiérrez *et al.*, 2008)**.

2.2. Chamomile

Is one of the most ancient medicinal herbs. It is a member of *Asteraceae/Compositae* family and represented by two common varieties German chamomile (*Chamomilla recutita*) and Roman Chamomile (*Chamaemelum nobile*). Chamomile are commonly used for many human ailments such as hay fever, inflammation, menstrual disorders, insomnia, ulcers, wounds, gastrointestinal disorders. Essential oils of chamomile are used extensively in cosmetics. Dried flowers of are used in the preparation of tea, which is consumed at a rate of more than a million cups per day. The beneficial effects of chamomile are related to the presence of several flavonoids **(Bhaskaran *et al.*, 2010)**. Other principal components are mucins, coumarins, phenol carboxylic acids

(phenyl substituted carboxylic acids), amino acids, phytosterols, choline, and mineral substances **(Ghizlane and Aziz, 2016)**.

2.2.1. Traditional usage

Externally, chamomile has been used to ear and eye infections, nasal inflammation. Chamomile is widely used to treat inflammations of the skin and mucous membranes, and for various bacterial infections of the skin, oral cavity and gums, and respiratory tract. Chamomile in the form of an aqueous extract has been frequently used as a mild sedative to calm nerves and reduce anxiety, to treat hysteria, nightmares, insomnia and other sleep problems. Chamomile has been valued as a digestive relaxant and has been used to treat various gastrointestinal disturbances **(Srivastava *et al.*, 2010)**.

2.2.2. Colic/Diarrhea

Chamomile extract may help shorten the course of diarrhea in children as well as relive symptoms associated with the condition, tow clinical trials have evaluated the efficacy of chamomile for the treatment of colic in children. Chamomile tea was combined with other herbs like be fennel and balm mint **(Srivastava *et al.*, 2010)**.

2.2.3. Anti-inflammatory

The flowers of chamomile contain 1–2% volatile oils including alpha-bisabolol, alpha-bisabolol oxides A & B, and matricin and other flavonoids which possess anti-inflammatory and antiphlogistic properties. Inflammation is associated with many gastrointestinal disorders complaints, such as esophageal reflux, diverticular disease, and inflammatory disease Studies in preclinical models suggest that chamomile inhibits Helicobacter pylori, the bacteria that contribute to stomach ulcers **(Srivastava *et al.*, 2010).**

2.2.4. Anticancer

In a recently conducted study, chamomile extracts were shown to cause minimal growth inhibitory effects on normal cells, but showed significant reductions in cell viability in various human cancer cell lines. A mixture of seven standardized botanical extracts including chamomile has been recently tested. The results confirm it to have a good safety profile with significant anticancer activities against androgen-refractory human prostate cancer, both in vitro and in vivo situation (**Srivastava *et al.*, 2010)**.

2.2.5. Common cold

Common cold (acute viral nasopharyngitis) is the most common human disease. It is a mild viral infectious disease of the upper respiratory system. Typically, common cold Is not life-threatening, although its complications (such as pneumonia) can lead to death, if not properly treated. Studies indicate that inhaling steam with chamomile extract has been helpful in common cold symptoms **(Srivastava *et al.*, 2010)**.

2.2.6. Diabetes

Studies suggest that chamomile ameliorates hyperglycemia and diabetic complications by suppressing blood sugar levels, increasing liver glycogen storage and inhibition of sorbitol in the human erythrocytes. The pharmacological activity of chamomile extract has shown to be independent of insulin secretion **(Srivastava *et al.*, 2010).**

2.2.7. Health Promotion

It has been claimed that consumption of chamomile tea boosts the immune system and helps fight infections associated with colds. The health promoting benefits of chamomile was assessed in a study which involved fourteen volunteers who each drank five cups of the herbal tea daily for two consecutive weeks. Daily urine samples were taken and tested throughout the study, both before and after drinking chamomile tea. Drinking chamomile was associated with a significant increase in urinary levels of hippurate and glycine, which have been associated with increased antibacterial

activity. In another study, chamomile relieved hypertensive symptoms and decreased the systolic blood pressure significantly increasing urinary output **(Srivastava *et al.*, 2010).**

2.2.8. Hemorrhoids

Studies suggest that chamomile ointment may improve hemorrhoids **(Srivastava *et al.*, 2010).**

2.2.9. Dental and Gingival health

Combination products with Chamomile as tooth paste or oral rinse, have demonstrated beneficial effects on the status of oral hygiene **(Pourabbas and Delazar, 2010).**

2.3. Rosemary

It is a plant belonging to the family *Lamiaceae* and originated from the Mediterranean region. However, it could be found all over the world. It is a perennial and aromatic plant, shrub-shaped with branches full of leaves, having a height of up to two meters and green leaves that exude a characteristic fragrance **(De Oliveira *et al.*, 2019).** Nowadays, rosemary one of the most popular sources of natural bioactive compounds, due to it is various pharmacological activities such as antibacterial, anti-diabetic, anti-inflammatory, antitumor and antioxidant **(Andrade *et al.*, 2018**). *Rosmarinus officinalis, L.* is a rich source of phenolic compounds and their properties are derived from its extracts and essential oils. Both are used for the treatment of illnesses and in the food preservation. In addition to the volatile constituents, extracts of rosemary also contain several antioxidant components, which belong mainly to the classes of phenolic acids and flavonoids **(Nieto *et al.*, 2018)**. *R. officinalis* is mainly composed of phenolic compounds, di- and triterpenes and essential oils. Polyphenols are antioxidant chemical compounds primarily responsible for the fruit coloring, which are classified as phenolic acids, flavonoids and nonflavonoids. In addition to their antioxidant properties, they play a very important role in the plant defenses against herbivores,

pathogens and predators; therefore, they have an application in the control of infectious agents in humans. In *R. officinalis*, the most common polyphenols are apigenin, diosmin, luteolin, and phenolic acids, especially rosmarinic acid, chlorogenic acid and caffeic acid, camphor, ursolic acid, betulinic acid, carnosic acid and carnosol **(Andrade *et al.*, 2018)**. By using disc diffusion, essential oils in rosemary tested are active against all the clinical strains from Escherichia coli strongly. Other studies have shown the antibacterial activity of rosemary oil against *bacillus cereus*, *Staphylococcus aureus*, *Salmonella choleraesuis*. and This essential oil was incorporated into meat reporting antibacterial activity against *Brochothrix thermosphacta* and *Enterobacteriaceae.* The inhibitory effect of rosemary is the result of the action of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol and isorosmanol. They interact with the cell membrane, causing changes in genetic material and nutrients, altering the transport of electrons, leakage of cellular components and production changes in fatty acid. Vegara *et al*. reported that the effectiveness of carnosic acid against pathogenic bacteria is superior to that of any other major extract component, including rosmarinic acid. Wong et al. concluded that the components of rosemary participate in the regeneration of α-tocopherol, which can be used as substitutes for vitamin C to enhance the stability of vitamin E **(Nieto *et al.*, 2018)**.

2.4. Staphylococcus aureus

Staphylococcus aureus produces a wide variety of exoproteins that contribute to its ability to colonize and cause disease in mammalian hosts. Staphylococcus aureus is a Gram-positive coccus about 1 μm in diameter. The cocci are usually arranged in grapelike clusters. The organisms are nonsporing, non-motile and usually non-capsulate. When grown on many types of agar for 24 h at 37°C, individual colonies are circular, 2–3 mm in diameter, with a smooth, shiny surface, colonies appear

opaque and are often pigmented (golden-yellow, hence the ‘aureus’). The main distinctive diagnostic features of *Staphylococcus aureus* Production of an extracellular enzyme, coagulase, which converts plasma fibrinogen into fibrin, aided by an activator present in plasma, Production of thermostable nucleases that breakdown DNA and Production of a surface-associated protein known as clumping factor or bound coagulase that reacts with fibrinogen **(Dinges *et al.*, 2000).**

Chapter Three

3. Materials and Methods

3.1. Study design

This was descriptive, cross-sectional laboratory based study.

3.2. Study area

Collage of Agricultural Studies, Sudan University of Science and Technology.

3.3. Study population

During this study, we targeted 13 of senior students (4^{th} and 5^{th} years) studying at Faculty of Agricultural Studies, Sudan University of Science and Technology. In the age group 20-25 and placed them in one room under the same conditions.

3.4. Preparation of plant samples for panel test (to make tea)

The dried herbs were collected from local market (Khartoum State). Guava leafs obtained from tree, then dried under room temperature (25°C) for one day to preparation of the aqueous extract 10 g of each type of the herbs sample was soaked in 70ml hot distilled water and left till cooled down with continuous stirring at room temperature. The plant herbs where divided into six group (A to F) and then an appropriate amount of each group (one calyces) was placed in an appropriate amount of water (3calyces) in a suitable container and amount of sugar was added 20% then the container was placed in the fire until it boiled. After that filtered and bottled then subjected to physiochemical and sensory studies to see how well consumers accept herbal teas.

3.5. Sensory evaluates

Administered questionnaire was used for sensory evaluating the data (Appendix). Samples of tea were coxed (A-F) were A=Guava, B=Rosemary, C=Chamomile, D= (homogenized sample 50%Guava\25% of two other herbs), E= (homogenized sample 50%Rosemary\25% of two other herbs) F= (homogenized sample 50%Chamomile\25% of two other herbs).

3.6. Development of questionnaire

Were written in Arabic for ease of understanding to ensure that accurate information is obtained. The questionnaire was designed to test the knowledge, sensory attribute and acceptability of the study subjects.

The samples were given numbers for evaluation and the numbers were from 1 to 4 (excellent –very good –good and acceptable) respectively, to find out the degree of acceptance of teas.

3.7. Statistical analysis

The collected data was categorized, coded and entered into the computer. Analysis of variance was done using the Statistix eight application.

3.8. Source of bacterial isolates

The clinical isolates were collected from College of Medical Laboratory in the Sudan University of Science and Technology during the period of February-2020.

3.9. Antimicrobials susceptibility of plant extracts (Cup-diffusion method)

Under aseptic conditions, preparation of glass petri-dish and put *the S. aureus* in Staph. Media, then three pores were made and swap the bacteria on dish. Then 1 ml of each herbs were poured into pores. Then then, the dish was kept into incubator, of 30°C for 24 hours.

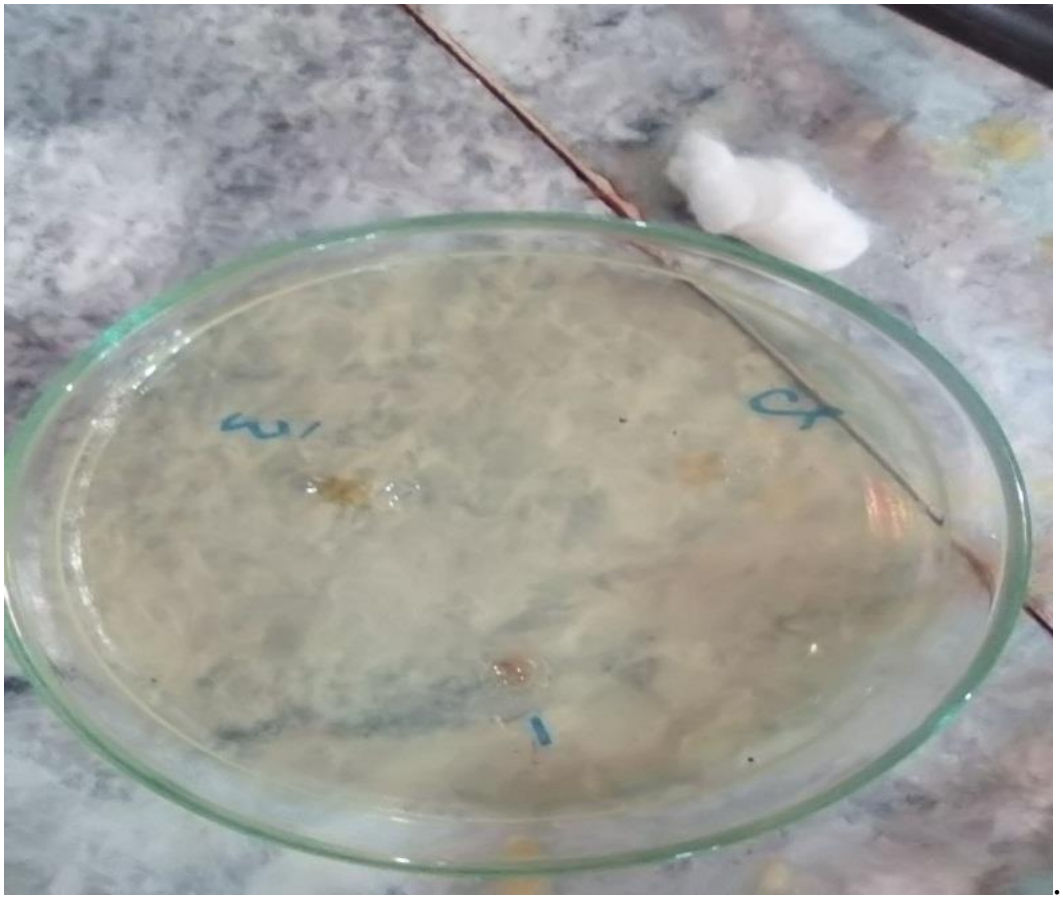

Figure (1): *S. aureus* incubated with three types of herb tea

The photo illustrates poured 1 ml of solutions in three pores on glass petri-dish marked by number from 1 to 3, when 1-3 show the extraction of guava leaf, rosemary and chamomile respectively, after swap the *staphylococcus aureus* into. After incubation we compare the antibacterial effectiveness according to the inhibition zone around pores. When the zone is big and heavy that mean the solution influent and inhibit microbial growth **(Londonkar and Ranirukmini, 2010, Gonçalves *et al.*, 2008, Rojas *et al.*, 2006).**

Chapter Four

4. Results and Discussion

4.1. Sensory evaluation of types of herbal tea

As shown in table (4.1) sensory evaluation revealed that there was not significantly different from one another for odor attribute, similarity trend was also reported by **AHMED (2018).** In relation to taste there a similarity between five samples forming two groups, the first group (A, B and C samples), second group (E and F samples), while the D sample is forming different category showed that higher significant.

Regarding the color similarity between (D, F and C samples) in one side, (E and A samples) in another side, and B sample is rank for a higher significant among all samples. For overall acceptability D sample had the best outcome.

4.2. Antimicrobial effect of the herbs on *S. aureus*

The search for antimicrobials has received a lot from natural sources Attention and efforts have been made to identify possible vehicles Acts as an antimicrobial agent suitable to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype for the development of less toxic and more effective medications to control growth of microorganisms. These compounds are of great importance Therapeutic application against human pathogens including bacteria, Fungi or virus. Several studies were conducted with extracts from different plants, and check the activity of antimicrobials as well To discover new antimicrobial companies **(Sen and Batra, 2012).**

At the present study we found antibacterial properties of guava leaf's extract, clinically proven to be able to inhibit *Staphylococcus aureus* growth by cup diffusion method used by many researchers **(Sen and Batra, 2012; Londonkar and Ranirukmini, 2010; Gonçalves *et al.*, 2008; El Astal *et al.*, 2005)**. This result

agreed with the results of **Naseer *et al.*(2018) and Vieira *et al.(* 2001)** who have also reported the antibacterial effect of guava leaves extracts and found that they inhibited the growth of the *S. aureus*. **Gnan and Demello (1999)** testing guava leaf extract found good antimicrobial activity against nine different strains of *Staphylococcus aureus* this may be due to the chemical properties found in guava leaves and various phenolic compounds and their anti-microbial properties. *Matricaria Chamomilla* is a well-known ingredient in alternative medicine. It has been intended for numerous purposes from dermatological to gastrointestinal and even neurological and psychiatry. Chamomile is known to possess anti-inflammatory, anti-oxidant and anti-bacterial effects. In the present study, we demonstrated that chamomile inhibits staphylococcus aureus growth by cup diffusion method. **(Alireza, 2012)**, test Antibacterial activity of the chamomile oil was tested against Gram-positive bacterial strains such as *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus subsp*. *Aureus*, using disc agar diffusion method. The Roman chamomile oil exhibited antioxidant activity according to **(Piccaglia *et al.*, 1993)** study. **(McKay and Blumberg, 2006)** Reported that Chamomile has moderate antimicrobial activity. The antimicrobial activity of chamomile essential oil was tested using the agar diffusion method wherein the essential oil has shown antibacterial effect with the inhibition zone on *Staphylococcus aureus* **(Stanojevic *et al.*, 2016***)***.* Several studies have reported that rosemary extracts show biological bioactivities such as hepatic protective, antifungal, insecticide, antioxidant and antibacterial. It is well known that the biological properties in rosemary mainly due to phenolic compounds. However, it is essential to take into account that these biological properties depend on different aspects. Their use in foods is limited because of their odor, color and taste. For that reason, commercial methods have been developed for the preparation of odorless and colorless antioxidant compounds from rosemary. This review gives a view on

the use of natural extract from rosemary to measure effect on *staphylococcus aures* activities, and we found a limit inhibition of bacteria growth. Other studies have shown the antibacterial activity of rosemary oil against *E. coli, Bacillus cereus, Staphylococcus aureus, Clostridium perfringens, aeromonas hydrophila, Bacillus cereus and Salmonella choleraesuis Gema* **(Nieto *et al.*, 2018, Burt, 2004)**. **(Zaouali *et al.*, 2010)** reported that antimicrobial activity of rosemary with *S. aureus*, improves with the presence of α -pinene as a major component. Gomez-Estaca *et al.* **(Gómez-Estaca *et al.*, 2010)** reported that rosemary oil inhibited the growth of common food bacteria contributing to food spoilage. **(Vegara *et al.*, 2011)** reported that the effectiveness of carnosic acid and rosmarinic acid against pathogenic bacteria. a mixture of *Rosmarinus officinalis, Salvia lavandulifolia and Thymus mastichina*. Their inhibitory and bactericidal activities in vitro against *Escherichia coli* and *Staphylococcus aureus* **(Vegara *et al.*, 2011).** It was observed that the antimicrobial effect of plant extract varies from one plant to another in different researches carried out in different regions of the world. This may be due to many factors such as, the effect of climate, soil composition, age and vegetation cycle stage, on the quality, quantity and composition of extracted product, different bacterial strains **(Masotti *et al.*, 2003).**

Health foundations have to increase their funding of these studies and research to help saving the lives of many peoples. This will also offer a great help in facing the emergence spread of antimicrobial resistance.

As shown in figure (2) the below photo illustrate the result of the experiment after removing the dish from the incubator after staying overnight. The heavy inhibition zone is observed around the pore of sample one (guava leaves tea). The zone of inhibition of sample two (rosemary leaves tea) is less heavy than guava leaves tea, and sample three (chamomile tea) comes after that.

Accordingly, and depending on cup diffusion method, this means that guava leaf is most influential on bacterial growth (antimicrobial) compared to rosemary and chamomile extract**.**

Table (4.1.): Sensory evaluation of the three herbal teas

Sample	Taste	Odor	Color	Overall acceptability
A	2.8667^{ab} ±1.0601	3.0000^{a} ±1.0000	2.0000^{b} ±0.8452	2.6000^{ab} ±1.1212
B	2.6667^{ab} ±1.0465	2.9333^{a} ±1.1629	3.0000^{a} ±1.1339	2.8667^{ab} ±0.9155
C	2.9333^{ab} ±0.9612	2.6667^{a} ±0.9759	2.5333^{ab} ±0.9155	3.0000^{ab} ±0.9258
D	3.3333^{a} ±1.1127	2.8000^{a} ±1.1464	2.6667^{ab} ±0.9759	3.0667^{a} ±0.8837
E	2.4667^{b} ±0.9904	2.8667^{a} ±1.0601	2.1333^{b} ±0.8338	2.4000^{b} ±0.7368
F	2.3333^{b} ±1.0465	2.5333^{a} ±0.9155	2.4000^{ab} ±0.9103	2.6000^{ab} ±0.7368

Mean± SD values bearing same superscripts for each quality attribute are not significantly difference ($p \leq 0.05$).

SD= Standard deviation.

A=100% Guava leaves extraction.

B=100% Rosemary extraction.

C=100% Chamomile extraction.

D=50%Guava, 25%Rosemary, 25%Chamomile.

E=25%Guava, 50%Rosemary, 25%Chamomile.

F=25%Guava, 25%Rosemary.

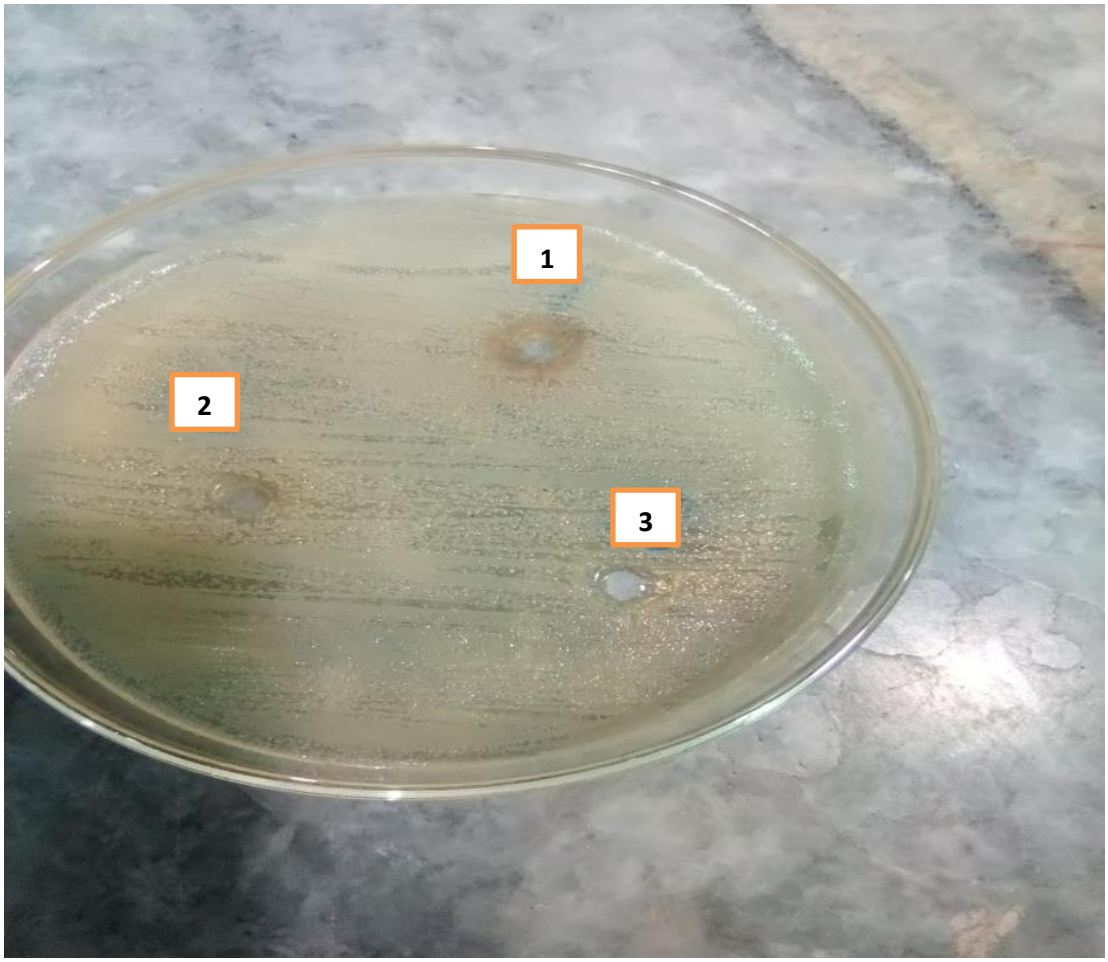

Figure (2): *S. aureus* inoculation final result (antimicrobial effect)

1 = Guava leaves tea.

2 = Rosemary leaves tea.

3 = Chamomile tea.

Chapter Five

5. Conclusions and Recommendations

5.1. Conclusions:

The following conclusions could be drawn:

- Guava leaf's extract showed highly antimicrobial activity against *staph. auras* compare with another two herb study.
- Guava extract tea obtained acceptance from the participants.

5.2. Recommendations:

1. It is recommended that the three types of tea can be used for tea made and also these herbal teas can be used as healthy food products.
2. Further studies are recommended to see the effect of these herbal plant extract (as tea) on other pathogenic microbes.
3. More in-depth studies are recommended to know the toxic effect of these plants and their associated side effects.
4. More researches is being done to increase the effectiveness of the pathogen anti-microbial agent in chamomile and rosemary.

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(Appendix)

جامعة السودان للعلوم والتكنولوجيا - كلية الدراسات الزراعية

قسم علوم وتكنولوجيا الأغذيه

استمارة تقييم حسي لمنتج شاي عشبي

النوع

ذكر ☐ أنثي ☐

الفئة العمرية

25-20 ☐ **30-26** ☐ **31 - فأكثر** ☐

الرجاء وضع علامة ( ) أمام كل خيار

العينة	التقييم	الطعم	الرائحة	اللون	التقييم الكلي
أ	ممتاز (1)				
	جيد جدا (2)				
	جيد (3)				
	مقبول (4)				
ب	ممتاز (1)				
	جيد جدا (2)				
	جيد (3)				
	مقبول (4)				
ج	ممتاز (1)				
	جيد جدا (2)				
	جيد (3)				
	مقبول (4)				
د	(1)ممتاز				
	(2)جيد جدا				
	(3)جيد				
	(4)مقبول				
ه	(1)ممتاز				
	(2)جيد جدا				
	(3)جيد				
	(4)مقبول				
و	(1)ممتاز				
	(2)جيد جدا				
	(3)جيد				
	(4)مقبول				