IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 18, Issue 4 Ser. I (July. – August. 2023), PP 21-25
www.Iosrjournals.Org

Evaluation of the inhibitory effect of some plant extracts on *Staphylococcus aureus*

Laila M. Alfageih[1], Sana A. Badr[2]
[1](Department of Medical science/Faculty of Dentistry / University of Tobruk, Tobruk, Libya)
[2](Department of Biochemistry/ Faculty of pharmacy/ University of Tobruk, Tobruk, Libya)
**Corresponding Author: Laila Alfageih*

Abstract:
This study aimed to investigate the inhibitory activity of the active substances of aqueous, alcoholic and acetonic extracts of plants (Hibiscus subdariffa , Artemisia herba – alba ,ZingiberofficinaleRosc and Salvia officinalis) and demonstrate their inhibitory effect on the growth of Staphylococcus aureus , For its clinical significance with its traits that cause urinary tract infection. The study also included the identification of the lowest effective concentrations of antibiotics Minimum Inhibitory Concentration(MIC) by using method (Kurby-Bauer Sensitivity Test) It was a combination (Gentamycin 25mg and Clindamycin 100mg) Alone high inhibitory effects when diluted 10^{-4}*,The penicillin group showed a lower inhibitory effect, and the rest of the antibiotics had no significant effects. The results of the plant extracts studied in this study were shown, that the Hibiscus subdariffa extracts had significant effects, as it reached the highest diameter of inhibition for bacteria Staphylococcus aureus 2.8 cm at a concentration of 100 g/200 ml in hot acetone and aqueous extract.The inhibition rate decreased by half upon dilution* 10^{-2}*. This does not negate that some extracts have given an inhibitory effect on bacteria, which indicates that these plants contain antibacterial growth substances, and in order to benefit from these plants, the components must be separated, identified and further studies conducted on them.*

Key words:*Staphylococcus aureus, Hibiscus subdariffa , Artemisia herba – alba ,ZingiberofficinaleRosc and Salvia officinalis , Minimum Inhibitory Concentration(MIC),Inhibitory efficacy , Antibiotics.*

Date of Submission: 08-07-2023 Date of Acceptance: 18-07-2023

I. Introduction

Despite the unprecedented development in the field of medicine, many infectious diseases still pose a threat to human health through the emergence of many resistant strains, and this may be attributed to the indiscriminate use of antibiotics [1].Hence the inefficiency of these antibiotics [2]. Infection with the bacterium *Staphylococcus aureus* is one of the diseases that affect the urinary tract and develop into other chronic diseases [3].Because of the emergence of resistance to traditional antibiotic treatment of these bacteria, it was necessary to search for new compounds with antibacterial effect derived from plants, probiotics and dietary supplements that act as natural substitutes without side effects and act as stimulants of the body's immune system. , As well as they are safe, inexpensive and effective remedies used against *Staphylococcus aureus* [4-5-6]. The current study included some medicinal plants, namely:

1- Hibiscus subdariffa, it is a member of the family Malvaceae, the plant contains some acids and active substances, namely: Tartaric acid, malic acid, vitamin C, Calcium oxalate and Tannins. Hibiscus is one of the laxative plants, like most Malvaceae plants, is considered a diuretic plant, and a source of vitamin C, and is used as a treatment to reduce blood pressure. In all cases, the treatment is taken in the form of syrup [7].

2-Artemisia herba _ alba: It is a member of the family Compositceae The Artemisia herba plant contains active substances, namely volatile oils contain the following active substances : the Santonin and a group of sterols (beta-sitosterol&stigmasterol). The Artemisia herba – alba plant has the property of lowering blood sugar in people with high blood sugar, and the substance santonin extracted from unopened inflorescences is useful in treating ringworms and is considered a stimulant. The leaf powder is used as an inhalation to treat cold [8-9].

3-Salvia officinalis Linn. : It is a member of the Lamiaceae family the plant contains the following active substances: Tannins,Terbenehydrocarbons, alpha & beta follone.The decoction of the plant is considered useful for vertigo (dizziness), nerve disorder, diuretic, and stimulant and useful for treating colds [10]

4- ZingiberofficinaleRosc: the plant contains the following active substances: Camphene, Linalol, resinous materials, starch and Gingerol. The plant is used to get rid of gases in the stomach and intestines, tonic and laxative. The plant is attributed to the treatment of tuberculosis and plague in ancient times, and to treat colds, expectant and reduce cough [11-12].

II. Material and Methods

a- Bacteria isolation:

Staphylococcus aureus bacteria were isolated by taking a sample of urine from patients with urinary tract infection and using a Sterilized Loop inoculated by planning method on a medium mannitol salt agarand suitable for the diagnosis of this type of bacteria, and the dishes were incubated at a temperature of 37 °C for a period of 24-48 hours. Theoretical and biochemical methods were adopted in diagnosing bacteria and confirming their diagnosis by molecular methods. Then, the inhibitory activity of the plant extracts used in the study was tested on the growth of Staphylococcus aureus in comparison with some antibiotics.

B-Preparation of antibiotics and sensitivity test:

Kirby & Bauer Sensitivity Test (K-B) modified method was used in the sensitivity test which is the method of spreading or inhibition around the disc and prescribed by the [13], Which is widely used around the world as a standard method in clinical laboratories after being approved by many organizations in the world such as the Food and Drug Administration (FDH) in America. [14-15].

For this study, a group of ideal antibiotics was selected, which have fewer side effects, and are available, where the following work was done:-

● 100 mg of each type of dry antibiotic was weighed by a sensitive scale and the weighed amount was placed in a sterile tube after writing the name of the antibiotic on it, then 1 ml of sterile distilled water for injection from (Chemothera.S.A-Belgium) was added and dissolved well.

● Decimal dilutions of the concentration prepared in the previous step were made as follows: - 1/10, 1/100, 1/1000, 1/10000 by transferring 0.1 ml of the previous concentration to 0.9 ml of sterile distilled water so that the dilution becomes 1/10 and so on. The dilution is 1/10000.

● Put 25 sterile and dry filter paper tablets in the tubes containing antibiotics used in the study. Then all the tubes were placed in the fridge for 24-48 hours to ensure that the tablets were saturated with antibiotics the amount in each tablet was calculated by weighing the tablet with the antidote minus its weight while it is empty (dry).

● a sterile tube with sterile distilled water free of any antibiotic was also taken and sterile filter paper tablets were placed in it as a Control.

After isolation and identification of the pathogen, it is cultured on a medium Sensitivity Test Agar (D.S.T.) then immediately followed by the placement of tablets saturated with the antibiotic 6 tablets per petri dish.

These antibiotic discs are distributed regularly on each plate, taking into account the non-interference of these antibiotics, especially when given a positive result, which may interfere with another inhibitory diskand in the middle a control disk is placed, then these dishes are incubated at a temperature of 37 °C for 18-24 hours, after which the clear areas around the antibiotic disk are examined with the naked eye, then their diameters are measured with a ruler in centimeters from the back of the dish or inside it, according to the state of inhibition, and this is considered the positive result for this examination, After referring to the tables for this method to judge the result and the extent of sensitivity, the negative result is the absence of impressive inhibition zones around the antibiotic disk or simple inhibition around the disk with the growth of a few colonies inside the inhibition zone is usually neglected and a result is given as resistance to that antibiotic.

C-Preparation of aqueous, alcoholic and acetone crude extracts of medicinal plants used in this study:

In this study, plant extracts from dry specimens was used which was collected from a fixed commercial source after confirming its type at the Agricultural Research Center. The plants samples were ground in the mill, after that four weights were taken at a rate of 100 grams and placed in glass flasks and the following organic and aqueous solvents were added to it.

● First flask 200 ml acetone 99.5% (Riedel-dettaen/Germany).

● Second flask 200 ml absolute ethanol 96% (BDH / U.K.).

● Third flask 200 ml sterile distilled water kept at room temperature 22 °C.

● Fourth flask 200 ml sterile distilled water kept at boiling temperature 100 °C.

● the pH of all the extracts was measured, and then left to soak for 24 hours at room temperature with occasional shaking, then filtered by sterile medical gauze in sterile glass dishes and placed in the incubator at 37 °C for 24-48 hours to dry the water and organic solvents.

Sterile filter paper discs were placed in it until it became saturated to obtain concentrated tablets. The weight of the dissolved substance in one disc was calculated by subtracting the empty disc from the saturated

disc. Then the concentrated saturated discs were transferred to sterile tubes.After that, a dilution of these extracts was made after drying, where the samples were weighed (100 mg / 1 ml sterile distilled water), placed in sterile tubes, and sterile filter paper discs were placed in them for 24 hours in the refrigerator, after which a test of the inhibition effectiveness of the extract on the bacteria used in This study is in the same way as the sensitivity test mentioned for antibiotics. (Al-Sulltan , 1993).

D- Statistical analysis

An analysis of variance (ANOVA) was performed, and the least significant difference (LSD) test was used to test the significance of the differences between the means.

III. Results

● Effect of antibiotics selected in the study and their decimal dilutions on *Staphylococcus aureus.*

The decimal dilutions used in the experiment are $10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}$, of 100 mg / 1ml of concentrated antibiotic used for this type of bacteria and thus use the lowest concentration of antibiotic that gives the highest inhibitory concentration (MIC).

The most antibiotics affecting the growth of bacteria Staphylococcus aureus are Clindamycin (Gent 25mg +Clind 100mg) they have high significant effects, followed very closely by the following antibioticsCloxacillin, Flucloxacillin, Gentamicin, Lincomycin, Penicillin G , (Genta 50mg + Clind 100mg), Benzyl pencillin, (Clind 50mg +Genta 100mg) respectively.

Then the following antibiotics came with the least effects compared to the above-mentioned antibiotics, respectively Bactrim, Cephalexin, Amoxicillin, and Ampicillin. Antibiotics that have no effect when this dilution 10^{-4} are Tetracycline and Erythromycin (Fig. 1).

As for the other dilutions, and despite the presence of very high inhibition zones in the high dilutions, such as the antibiotics Cloxacillin, Flucloxacillin, Lincomycin, Clindamycin, Gentamicin and Cephalexin, respectively, had the same inhibitory effect against bacteria at the level of the other decimal dilution and even the highest dilution as previously mentioned. Except for the antibiotic Bactrim, which gave a high zone of inhibition compared with Gentamicin, Cephalexin at high concentrations only, especially at the concentration (100 mg / 1 ml) compared to other concentrations (Fig. 1).

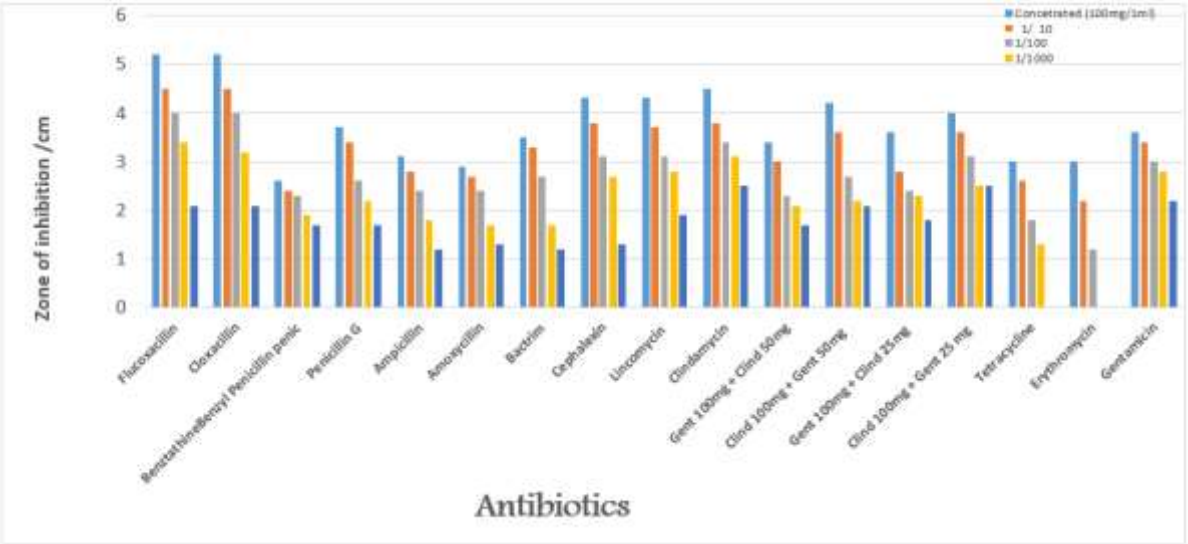

Figure 1: Shows the sensitivity of *Staphylococcus aureus*to some antibiotics and their decimal dilutions.

●The results obtained from the effects of aqueous, alcoholic and acetone-soaked raw extract of the following medicinal plants(*Artemisiaherba – alba,ZingiberofficinaleRosc, Hibiscus subdariffa* and *Salvia officinalis*) on *Staphylococcus aureus*bacteria studied in the study.

It is noted from (Fig. 2) that Hibiscus subdariffa extracts had significant moral effects compared to other plant extracts. The inhibition rate was halved at dilution 10^{-1} As for the ZingiberofficinaleRosc extract, it showed inhibition of bacteria less than the Hibiscus subdariffa at the level of half, except for the boiled aqueous extract, which did not give any result, noting that the aqueous ginger at a temperature of 22 ° C gave a significant and clear inhibition, which indicates the effect of the active substance against bacteria at high temperature. Also, the effectiveness of the alcoholic and acetone extracts disappeared when diluted by 10^{-1} or more, which indicates their weakness in the extract (Fig. 2).

As for Artemisia herba plant, the aqueous extract at a temperature of 22°C gave a significant and clear effect (P 0.05), and it decreased by half when boiling due to the effect of the antibacterial active substances on high temperature. It decreased by almost half when dilution as well (Fig.2).

It did not appear at all when diluted in boiled water, while the acetone extract showed a less inhibition effect than the aqueous extract. It also decreased when diluted 10^{-1}.

The alcoholic extract of *Artemisia herba* gave a clear inhibitory effect to the concentration used in the experiment, and when diluted 1-10, it did not show any clear inhibitory effect. All *Salvia officinalis* extracts gave inhibitory effects but less than those of *Hibiscus subdariffa* extracts (Fig. 2).

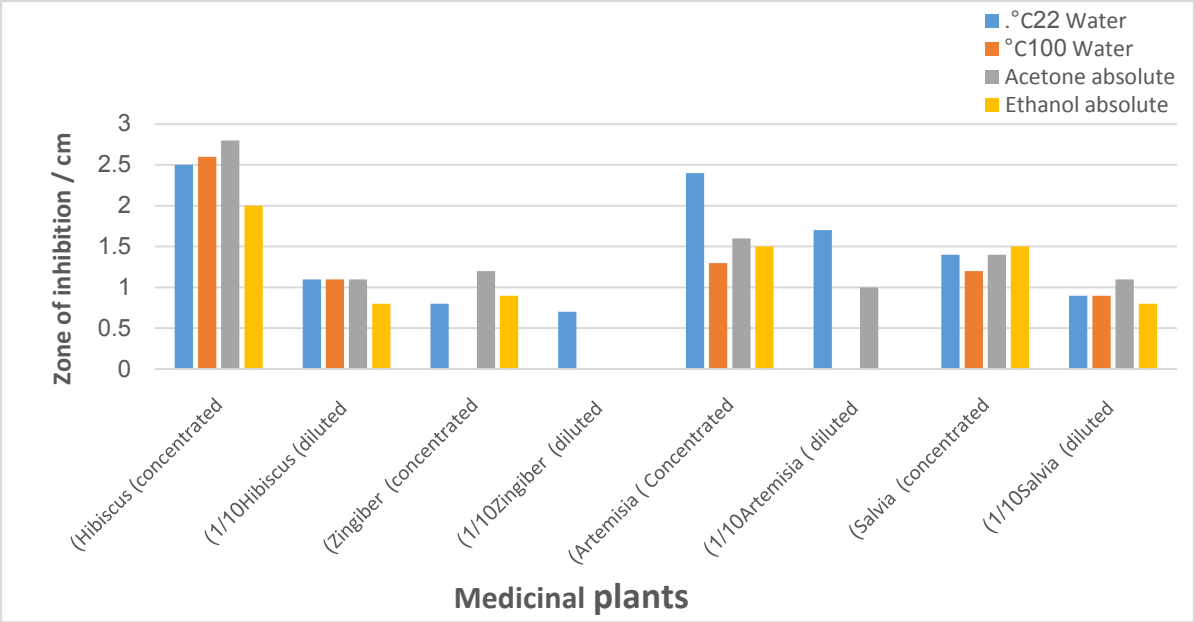

Figure (2): Shows the differences between the inhibitory effect of extracts of Artemisia , Zingiber , Hibiscus and Salvia raw aqueous, alcoholic and acetone concentrated 100 g / 200 ml on *Staphylococcus aureus*.

IV. Discussion

It was found that the aqueous, alcoholic and acetone extracts of plants with medicinal effect used in this study, namely Hibiscus subdariffa , Artemisia herba – alba ,ZingiberofficinaleRosc and Salvia officinalis, did not have high inhibitory effects on Staphylococcus aureus bacteria compared to those observed for many antibiotics, Where the effect of some of them did not exceed 50% of the inhibitory effect of antibiotics, which showed a significant effect on bacteria, Also, when comparing the inhibitory effect of extracts of synthetic plants on the growth of Staphylococcus aureus with the antibiotic that had the highest inhibitory effect on bacteria, it was found that there was a variation in the effect of different extracts for each type of plant tested. Some extracts of the Hibiscus subdariffa plant have had a remarkable effect exceeding 50% of the antibiotic effect on Staphylococcus aureus and it can be useful to use this plant in the treatment of these bacteria if the effectiveness of the compounds contained in the Hibiscus subdariffa plant can be increased after conducting the necessary studies on it. It can now be said that the herbal extracts studied in this research are very likely to contain more than one type of active compounds, which often work synergistically with each other, and the loss of the effectiveness of one of the components does not necessarily mean the complete loss of its effectiveness, but may reduce its effectiveness As happened to some Artemisia herba-alba extracts, for example, against Staphylococcus aureus (Fig. 2), the highest inhibitory activity appeared in the aqueous extract at a temperature of 22 ° C, but this effectiveness decreased significantly when prepared in boiling water, which may mean that some active compounds are affected by boiling water, while the Artemisia herba – alba extract is confirmed in water at a temperature of 22 ° C, which gave a higher effectiveness than the rest of the extracts, which was the least effective Artemisia herba – alba extract in boiling water 100 ° C, and this may be due to the lack of solubility of the components of the Artemisia herba – alba plant in organic solvents and affected by heat in the case of boiling water (Fig. 2).

Through the above, there is still a need for more detailed study on the possibility of using extracts of plant species tested in this study by trying to isolate the effective compounds and the possibility of purifying them and increasing their concentration to ensure their effectiveness against various pathogenic bacterial species, and determining the effective doses for each species in the laboratory as happened previous attempts for the same purpose [16-17-18].

References

[1]. Pillai, S. P., Pillai, C. A., Shankel, D. M., &Mitscher, L. A. (2001). The ability of certain antimutagenic agents to prevent development of antibiotic resistance. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 496(1-2), 61-73.

[2]. Tilgner, S., Crouse, V., Reiley, J., Buckman, L., Scandalios, B., &Fratz, D. (2000). Urinary tract health. Herbal Transitions V, 2..

[3]. C.C . Fuda; J.F . Fisher; and S. Mobashery, (2005). "Beta-lactam resistance in Staphylococcus aureus: the adaptive resistance of a plastic genome". Cell Mol. Life Sci. 62, 2617-2633.

[4]. Naidu, K. C., Lalam, R., &Bobbarala, V. (2009). Antimicrobial agents from Rubiacordifolia and Glycyrrhizaglabra against phytopathogens of Gossypium. International Journal of PharmTech Research, 1(4), 1512-1518.

[5]. S .D. Fihn (2003). "Acute uncomplicated urinary tract infection in women "The New England Journal of Medicine: 349(3):259-266.

[6]. S.S.R. . Challa; M . Kumar and Faruq, (2011) Adv. "Drug Delivery Reviews".

[7]. Shruthi, V. H., & Ramachandra, C. T. (2019). Roselle (Hibiscus sabdariffa L.) calyces: a potential source of natural color and its health benefits. In Food bioactives (pp. 169-190). Apple Academic Press.

[8]. Keith, H. G. (1965). A preliminary check list of Libyan flora. Government of the Libyan Arab Republic, Ministry of Agriculture and Agrarian Reform.

[9]. Adewumi, O. A., Singh, V., & Singh, G. (2020). Chemical composition, traditional uses and biological activities of artemisia species. Journal of Pharmacognosy and Phytochemistry, 9(5), 1124-1140

[10]. Ghorbani A, Esmaeilizadeh M. Pharmacological properties of Salvia officinalis and its components. J Tradit Complement Med. 2017 Jan 13;7(4):433-440. doi: 10.1016/j.jtcme.2016.12.014. PMID: 29034191; PMCID: PMC5634728.

[11]. Kotb fawzy (1985). Medicinal plants in Libya 1st publication Arab Encyclopedia House.

[12]. Bhaskar A, Kumari A, Singh M, Kumar S, Kumar S, Dabla A, Chaturvedi S, Yadav V, Chattopadhyay D, Prakash Dwivedi V. [6]-Gingerol exhibits potent anti-mycobacterial and immunomodulatory activity against tuberculosis. IntImmunopharmacol. 2020 Oct;87:106809. doi: 10.1016/j.intimp.2020.106809. Epub 2020 Jul 18. PMID: 32693356.

[13]. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Tech Bull Regist Med Technol. 1966 Mar;36(3):49-52. PMID: 5908210.

[14]. C. H. Collins, M. L. Fatricia& J. M. Grange (1995). Microbiological Methods, 7th edition. Butter worth Heinemann Publications 102- 360.

[15]. NCCLS (National Committee for Clinical Laboratory Standards) (U.S) Performance Standards for Antimicrobial Disc Susceptibility Tests, Approved Standard 3rd edition. Villanova, Pa: NCCLS, (1984). Vol – 4, No. 16.

[16]. M. C. Recio, J. L. Rios & A. Villar (1989). A review of some antimicrobial compounds isolated from medicinal plants reported in literature 1978- 1988. Phytotherapy. Res. 3 (4): 117 – 125.

[17]. B. Desta (1993). Ethiopian Traditional herbal drugs. Part 2 Antimicrobial activity of 63 medicinal plants. J. Ethnopharmacol, 39: 129- 139.

[18]. M. Al-Juwarey, A. Y. Shareef& B. A. Ahmed (1994). The Antibacterial bacterial isolates. J. Education & Science, 17: 75 – 84.