University of Thi-Qar Journal of Science (UTsci)
ISSN Onlin:2709-0256, ISSN Print: 1991-8690 Volume (8), No.1

Effectiveness study of Artemisia herba-alba and Borage officinalis leaf extract against bacteria *Staphylococcus aureus*

Raghad A. AL.Askary
Department of Biology / College of Sciences / University of Thi-Qar

Haider R. Malih
Department of Biology / College of Sciences / University of Thi-Qar

***Abstract*— The inhibitory efficacy of aqueous extract of *Artemisia herba-alba and Borago officinalis* leaves was evaluated a type of bacterium that posits a cram stain, including the isolated Staphylococcus bride the results showed efficacy against microbial for both extracts The bacteria are under study. Some of the active compounds have been detected in the leaves of *Artemisia herba-alba and Borago officinalis* These leaves were found to contain (tannins, alkaloids, resins, soaps, phenols, flavonoids and Glycoside). Also, the sensitivity of this isolation was studied for some antibiotics, and most of them were resistant to Ceftriaxone, Amikacin, Imipenem, Ciprofloxacin, with a rate of 100%. The results show Synergism efficacy when mixing the antibiotic Ceftriaxone, Amikacin, Imipenem, Ciprofloxacin with each of the aqueous extracts of the blocks *Artemisia herba-alba and Borago officinalis* on a different bacterium under study.**

Keywords— *Artemisia herba-alba and Borago officinalis*, Antimicrobial, Gram positive bacteria

I. INTRODUCTION

The use of plant extracts to treat many diseases has increased at present, especially after increasing the resistance of bacteria for pathogenic bacterials that cause a major problem for an individual's health, whether in developed or developing countries[1,2] ،studies have shown that *Staph. aureus* bacteria is one of the most pathogenic *staph* bacteria that can cause infection respiratory system in addition to *Streptococcus spp*. causes pneumonia and pharyngitis، it also caused meningitis, mastitis and urinary tract infections [4, 3]. for the treatment of these diseases, many of the antibacterial agents, but the increased use of these substances, which is often random, and for a long time, has led to the emergence of effects side harm to individual health on the one hand and the emergence of anti-bacterial strains on the other hand [5, 6] if the plant is contained on a group of active compounds that have been known since ancient times in the treatment of many diseases, as some studies have shown that some plants have wide physiological and pharmacological efficacy and as protection and preservation factors for foods, they are natural alternatives to chemicals used either in the pharmaceutical field or in the field of food preservation or prolonging its life when storing, using natural additives with antibacterial efficacy by food factory owners and food researchers, it is safe to use and side effects caused by less harmful compared to industrial drugs [7]. *Artemisia absinthium* is a herbaceous perennial plant with filamentous roots. The Stems are straight, evolving to 0.8-1.2 meters (2 feet 7 by 3 feet 11 inches) (now and then already over 1.5 meters, but occasionally) are long, serrated, branched, and silver-green [8] . The leaves are organized in a spiral shape, greenish-gray above them and white underneath, secured with luxurious silvery white trees, bearing small, oil-producing organs; The base takeoff length is 250 mm (9.8 in), and its length increases to triple with long petioles, with a smaller wiping (the ones on the stem), 50-100 mm (2.0-3.9 in) long, less separated, and with short petioles. tops take off can be clear and quiet (without stalk). Its flowers are pale yellow, tubular, and grouped in curved round heads (Capitola), which in turn congregate in branched, green foliage [9]. it is used for flavor in some spirits and other wines, including bitter, biscuit, wine, and bellenkovac. as a drug, it is used to treat poor digestion, as a way to counteract poor appetite, for various infectious diseases, crohn's disease, and immunoglobulin nephropathy [10]. In the Middle Ages, wormwood was used for med spices, and in Morocco it is used with tea, called chiba, in britain in the eighteenth century, wormwood was used in some cases instead of hops in beer [11] The borage plant (*Borago officinalis* L.), locally called Maui rose, is a herbaceous annual plant. the height of the plant is about (80 - 50) cm and is The plant is juicy with many hairs, the flowers are separate and standing, and the leaves are broad with a light blue color [13, 12]. spread out The plant is widely used in the Mediterranean countries, and it is native to Europe and South Africa [01] due to the nature of the chemical composition For borage plant, it has appeared to it many uses in the pharmaceutical and food industries, as it is one of the herbs used It is a spice in addition to being used in drinks and salads [1], as it is used in the treatment of many weaknesses physical illness, such as fever, is used to stimulate the heart and is used to treat respiratory diseases [2, 3]. Hence the aim of this study came to study the inhibitory efficacy of the aqueous extract of the borage plant *Borago officinalis* against some types of bacteria that are positive for gram negative stain.

Figure 1. Species *Borago officinalis* plant (Kapoor and Nair, 2005).

Figure . 2. Species *Artemisia herba-alba* plant (Shah, 2014).

II. MATERIALS AND METHODS

A. Collection and classification of study stations

The vegetable parts (leaves) used in this study were obtained from the local markets of Dhi Qar Governorate, and the plants were classified by specialists in the Iraqi national herb *Borago officinalis* and *Artemisia absinthum* and dried in the shade for two weeks and after drying it was crushed by the electric mill and then was Keeping samples in the department's laboratory.

B. Sources of isolation

Fourteen isolates were obtained from *Staph aureus* ، From Al-Hussein Teaching Hospital in Dhi Qar Governorate, which was isolated from cases of urinary tract infection during the month of September 2019 .The age of patients ranged between 18- 40 years.

C. Antibiotic sensitivity test

The Disk diffusion method mentioned in [14] is used to conduct an antibiotic sensitivity test Mueller Hinton agar medium included Ceftriaxone (30µg), Imipenem (10µg), Gentamicin (10µg), Amikacin (10ug). and Ciprofloxacin (10µg),(Bioanalysis, Turkey) were compared results with the standard tables mentioned in [14] to determine the diameter of the inhibition zone.

D. Detection of active compounds

The following methods have been followed to detect the active compounds:

1- Alkaloids: by using the method (6) (the process will be boiling) 0.1 ml of plant powder with (51) ml of distilled water acid (4%) of hydrochloric acid and the solution is filtered after cooling (0.5)ml is taken from the filtrate and a Wackner reagent is added,

2- 2- Flavonoides: using a method [7] consisting of two solutions The first solution is to dissolve (1) grams of the plant in ethyl alcohol (95%), while the second solution consists of (1) ml of alcohol Ethyl (51%) to (1) ml of potassium hydroxide solution (51%). When mixing the two solutions, the yellow color appears. on the presence of flavonoids,

3- Risine resins: by using the method (8) add (51) ml of ethyl alcohol (95%) to (5) g of plant powder and heated with a water bath for a period of (2) minutes, then it was filtered. (110) ml of distilled water was acidified and acidified. The hydrochloric and turbidity of the solution indicates the presence of resins,

4- Tannins: using method [8] (boiling process) 1 grams of plant powder in (51) ml of distilled water for several minutes, after which the filtration process is completed and the filtrate is divided into two parts added to The first part is a solution (0%) of lead acetate, and the presence of Tannins indicates a gelatinous precipitate, while adding to the second part solution (0%) of ferric chloride, green and blue indicate the presence of tannins, glycosides: Using a method [12] mixed parts of plant extracts with a benedict reagent and the appearance of a red precipitate sugars in these extracts,

5- saponine: by method [12], the aqueous solution of plant powder is strongly shaken. In a test tube, saponins are indicated with a thick foam, Phenols: using method [13].

E. Prepare plant extracts

The aqueous extract was obtained by dissolving (50) grams of each sample of the plant powder in (250) ml of distilled water. Soak for 24 hours at room temperature with continuous shaking by vibrator. It is filtered by a piece of medical gauze and then by filter paper. Then put it in the incubator 40 °C for a period of three days. After it dries, put it in the fridge 4 °C until use.

1) Preparation of Artemisia herba-alba borage extract (aqueous):

Stock solution stock solution prepared From each of the hot aqueous extract of the borage plant, as (1) g of each extract was transferred Separately to a flask with a capacity of (100) ml and complete the volume to (100) ml with distilled water and sterile using filters with a diameter of

0.22 Micrometer. Concentrations of 1000 ppm [15] were attended.

2) Study of the inhibitory activity of Artemisia herba-alba and borage extracts:

tested the sensitivity of the 14 isolates to extracts (aqueous) hot borage plant pollinated the surface of the dish containing the Mueller Hinton agar medium using a sterile cotton swab with a bacterial lung containing 10 * 1.58 colony / ml, drilled with a diameter of (5) mm on the surface of the culture medium by sterilizing the cork perforator and placing 50 mg of each extract separately.The dishes were incubated at 37 ° C for 24 hours, the efficacy of the extracts was determined by measuring the average diameter of the resulting inhibition area. Measured each hole in millimeters [16].

3) Study of mixing the antibiotic with the extract

Both the Amikacin and Ceftriaxone antibiotics were mixed with Artemisia Borago officinalis and the water (hot water) method was not followed using the chess board method, and as a result, a broker from Muller-Hunton Mueller Hinton agar and similar volume sizes in the inhibitory concentration assured me the selection test for each Antibacterial, calculation, extraction and calculation of what was mentioned in [17] and the FIC concentration factor was calculated. Chess by the formula:: -

$$FIC = \frac{\text{MIC of the antagonist or extract in the mixture}}{\text{MIC of the antagonist or extractor un}}$$

III. Results and Discussion

The results of the current study dealing with sensitivity to antibiotic bacteria, it was found that staph bacteria were resistant to all the antibiotics used under study, and no allergic activity will be shown against any of the antibiotics as shown in Table (1). It was found that *Staphylococcus* has resistance to gentamicin and ciprofloxacin. They agree with our findings, [1]. Urus was resistant to gentamicin and ciprofloxacin. Studies have shown that the cause of bacterial resistance to many antibacterial agents is due to several reasons, including a change in the permeability of the cytoplasmic membrane or a change in the target site where the antigen works, as well as the production of beta-lactamase crossover and Campbell's enzymes.

Table (1): Antibiotic susceptibility pattern of *Staph. aureus* isolated from UTI patients (*Staph. aureus* = 14 isolates)

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
CRO	0 (0.00)	0 (0.00)	14 (100.00)
AK	0 (0.00)	0 (0.00)	14 (100.00)
IMI	0 (0.00)	0 (0.00)	14 (100.00)
CIP	0 (0.00)	0 (0.00)	14 (100.00)
GEN	0 (0.00)	0 (0.00)	14 (100.00)

The results revealed the detection of active compounds according to the methods mentioned in the working methods of the aqueous extract leaves of Artemisia herba-alba Borago officinalis containing aqueous extract (Flavonoids Glycosides Tannins and alkaloids, resins, Saponins phenols, volatile oils) in Table (2), which shows the active substances present in both extracts, these results agreed with [18]

Table (2): Initial detection of active compounds and groups in the study plants

Effective compounds	Detection guide	*Borago officinalis*	*Artemisia herba-alba*
Flavonoids	Dark bluish color	+	+
Glycosides	Red precipitate	+	+
Saponins	Dense foam for a long time	+	+
Resins	Be turbid	+	+
Phenols	The appearance of a greenish-blue color	+	+
Alkaloids	The appearance of a white precipitate	-	-
Tannins	The appearance of a bluish green color	+	+
Volatile oils	Gray appearance	+	+

Sensitivity of bacteria to plant extract preparation of results: as in Table No. (3) *Staphylococcus* bacteria were 100% sensitive to *Artemisia* plant extract, while there was a slight change in the sensitivity of staph bacteria to *Bourge* plant extract by 14% and this is because it contains most of the active substances ingredients (phenol, flavonoids, tannins, glycosides, soaps and substances volatile oils) compared to the rest of the plant extracts that have been studied and can be recovered for this reason, as it has been proven that there are no alkaloids in water extracts in all plants and this is due to the fact that they are substances that require an amount of heat during extraction, or some of them are insoluble in water and soluble only in alcohol (ether, benzene) [19] Perhaps these staphylococci are sensitive to other plant extracts because glycosides consist of two parts: the first is soluble in water and the second is insoluble in sugar in alcohol [20] . As for phenols, their presence is due to the ability of these plants to kill and prevent. Many microorganisms, especially pathological ones [21]. Soap is observed in the aqueous extract of plants for all plants of study, because it dissolves in water and gives soap foam unlike the alcoholic extract, because the diabetic part is an essential part of its composition, so it is characterized by its ability to dissolve in water, not in alcohol, and this sugar is often glucose sugar [22].

Table (3): Sensitivity of plant Aqueous extract on *Staphylococcus aureus* isolates:

Plant extract	Sensitive (%)	Intermediate (%)	Resistant (%)
Artemisia	14 100.00)	0(0.00)	0(0.00)
Borage	2(14.30)	1 (7.10)	11 (78.60)

The antimicrobial activity of *Artemisia herba-alba* and antibiotics on staphylococci. Uros isolate inhibitory effect against bacteria when used in conjunction with antibiotics, which proves the synergistic action of this compound towards the growth of bacterial continuity.

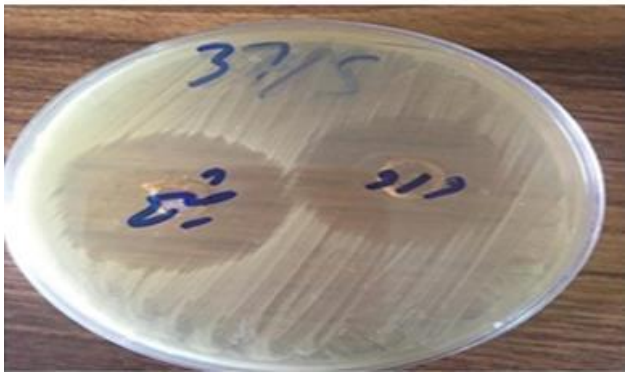

Figure (3): inhibition zone Sensitivity of bacteria to plant extract

Table (4): Antimicrobial susceptibility of *Artemisia herba-alba* and antibiotics on *Staph. aureus* isolates:

Plant extract with Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
***Artemisia herba-alba* with IMI**	10 (71.4)	2 (14.3)	2 (14.3)
***Artemisia herba-alba* with CIP**	12 (85.7)	2 (14.3)	0 (0.00)
***Artemisia herba-alba* with CRO**	11(78.6)	2 (14.3)	1(7.1)
***Artemisia herba-alba* with GEN**	12 (85.7)	2 (14.3)	0 (0.00)
***Artemisia herba-alba* with AK**	14(100.00)	0 (0.00)	0 (0.00)

Bacterial antibacterial activity and antibiotics on staphylococci. Isolates Urus The following table illustrates the synergistic action of bull flower with antibacterial agents against growing bacterial colonies where the effect of the resulting mixture on growth is shown. The results indicated that the bacteria showed an allergy to synergistic use of the mixture

Table (5): susceptibility of Streptococcus isolates to antibiotics

Plant extract with Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
B. officinalis with IMI	12 (85.7)	2 (14.3)	0 (0.00)
B. officinalis with CIP	10 (71.4)	2 (14.3)	2 (14.3)
B. officinalis with CRO	11(78.6)	1(7.1)	2(14.3)
B. officinalis with GEN	11(78.6)	1(7.1)	2(14.3)
B. officinalis with AK	13 (92.9)	1(7.1)	0 (0.00)

The effect of mixing plant extracts used in the study was studied at a concentration of (50) mg / ml with five types of antibiotics ceftriaxone, amikacin, imipenem, ciprofloxacin and gentamicin. on the isolates of *Staphy*. aureus bacteria, depending on their sensitivity and resistance to antibiotics, using the Muller-Hinton Agar tablet dispersion method for easy and rapid bacterial growth and pH stability, as well as a high penetration of antibiotics and plant extracts [23] as shown in Table (4. 5) The importance of plant extracts used in the study when mixed with antibiotics and their effect on the isolates of *Staphy*. aureus bacteria, especially antibiotic resistance, where plant extracts showed a cooperative effect against bacteria that were resistant to antibiotics. As for sensitive isolates, they showed a higher susceptibility to the synergistic effect, and this corresponds to [24] the synergistic efficacy when mixing antagonisms with equations is due to several reasons, including the mechanism of inhibiting cell growth through the influence on the synthesis of nucleic acid or protein, or its effect on the cell wall or energy sources, as the first compound works on The cell wall while the second compound works to stop the DNA, which results in an increase in the effectiveness of bacterial cell growth inhibition. The result agreed with [25]

REFERENCES

1. Wilson, C. L. and Droby, G.G. (2000). Microbial food contamination. Boca Raton, FL. USA: CRC Press.

2. Friedman, M.; Henika, R.P. and Mandrell, E.R. (2002). Bactericidal activities of plant essential oil and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, Listeria monocytogenes and Salmonella enteric. Journal of food protection. 65: 1545-1560.

3. Levinson, W. and Jawetz, E. (2000). Medical microbiology and immunology (Examination and board review), 6th. Singapore, New Delhi: 582 pp.

4. Todar, K. (2000). *Staphylococcus*, Todaŕson line textbook of bacteriology.

5. Lai, S.; Themblay,J. & Deziel, E. (2009). Swarming motility a multicellular behavior conferring antimicrobial resistance. Environmental microbiology. 11:126-136.

6. SCENIHR (Scientific Committee on Emerging and New Identified Health Risk) (2010). Research strategy to address the knowledge gaps on the antimicrobial resistance effects of biocides, .

7. Nychas, G.J.E. ; Tassou, C.C. and Skandamis P. (2003). Antimicrobial from herbs and spices .InS. M. Rollers (Ed.).Natural antimicrobials for the minimal processing of foods (pp.176-200). New York wood head publishers, CRC press.

8. Nadeem, A. S., Mujeeb, M., Amir, M., & Waseem, A. S. (2010). Impact of biodiversity on Umbelliferone content of Aegle marmelos (Rutaceae). Planta Medica,76(5):37pp..

9. Wani, H., Shah, S. A., & Banday, J. A. (2014). Chemical composition and antioxidant activity of the leaf essential oil of Artemisia absinthium growing wild in Kashmir, India. Aust J Pharm, 3(2):90-94pp

10. Ryu, J. C., Park, S. M., Hwangbo, M., Byun, S. H., Ku, S. K., Kim, Y. W., ... & Cho, I. J. (2013). Methanol extract of *Artemisia* apiacea hance attenuates the expression of inflammatory mediators via NF-κB inactivation. Evidence-Based Complementary and Alternative Medicine,

11. Msaada, K., Salem, N., Bachrouch, O., Bousselmi, S., Tammar, S., Alfaify, A., ... and Hammami, M. (2015). Chemical composition and antioxidant and antimicrobial activities of wormwood (*Artemisia absinthium L.*) essential oils and phenolics. Journal of Chemistry,

12.لشتشتيرن ، هيرمان (1981) . النباتات الطبية ترجمة ناصر حسين صقر. دار ابن الاثير للطباعة والنشر ، الموصل:صفحة 188 .

13. خليفة ، حسن (2009) . جنة الاعشاب ، الشامل للاعشاب المجربة . الطبعة الاولى ، دار الاسراء للنشر ، عمان– الاردن : صفحة الطبعة الاولى ، دار الاسراء للنشر ، عمان – الاردن : صفحة 359

14.CLSI (2012). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI document M 100-S22. Wayne, PA: Clinical and Laboratory Standards Institute.

15.Sato, J. ; Goto, K.; Nanjo, F.; Kowai, S. and Murata, K. (2000). Antifungal activity of plant extracts against Arthrinium *sacchari* and Chaetomium funicola . J.Biosci. Bioeng. 90(4):442-446.

16. Pavrez, M. ; Mahboob, H. K.; Zahuul, I. and Shek, M.H. (2005). Antibacterial activities of the petroleum ether, Methanol and acetone extracts of Kaempferia galangal. Rhizome. J. Life Earth Sci., 25-29.

17. Sibanda, T. and Okoh, A.I. (2008). In vitro evaluation of the interactions between acetone extracts of Garcinia kola seeds and some antibiotics. African Journal of Biotechnology.7 (11):1672-1678.

18. النعيمي ، حنان عدنان شاكر . (2115). تقييم فعالية بعض المستخلصات النباتية على نمو البكتريا المرضية الموجبة الصبغة المعزولة من حالات التهاب البلعوم واللوزتين . رسالة ماجستير . معهد الهندسةالوراثية والتقنيات الاحيائية للدراسات العليا . جامعة بغداد

19. Mawlood, S. I. (2011). Chemical and biological study of Iraqi Kurdistan chamomile flower (Matricaria recutita L). Baghdad Science Journal, 8(3), 736-74

20.Steiner, S. S., & Pohl, R. (2010). U.S. Patent No. 7,713,929. Washington, DC: U.S. Patent and Trademark Office.

21.Burden, R. S., Loeffler, R. S. T., Rowell, P. M., Bailey, J. A., and Kemp, M. S. (1986). Cyclodebneyol, a fungitoxic sesquiterpene from TNV infected Nicotiana debneyi. Phytochemistry, 25(7), 1607-1608

22. Belitz, H. D., Grosch, W., & Schieberle, P. (2004). Sugars, sugar alcohols and honey. In Food chemistry (pp. 862-891). Springer, Berlin, Heidelberg.

23. Scott, D. B., Farnden, K. J., & ROBERTSON, J. G. (1976). Ammonia assimilation in lupin nodules. Nature, 263(5579), 703-705.

24. Truppa, C., and Abo-Shehada, M. N. (2020). Antimicrobial resistance and conflict in the Middle East: A systematic review.

25. ناجي ، سيماء عمار. (2010). التاثيرات التازرية لبعض مستخلصات النباتية والمضادات الحيوية في بكتريا *Staohylococcus aureus*المعزولة من البلعوم الفمي . رسالة ماجستير، كلية التربية ابن الهيثم. جامعة بغداد.