Alcoholic Rosmarins Officinalis: Antibacterial Activity Aganist Local Isolates Staphylococcus Aureus

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Abstract

The current research included isolation and identification of the active compounds of rosemary plant. Soxhlet was used in the separation process using an alcoholic solvent (ethanol at a concentration of 70%). The detection and identification of the active compounds were accomplish out using gas chromatography - mass spectrometry.(Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-and 11-Octadecenoic acid, methyl ester and cis-13-Octadecenoic acid), and the antibacterial of the alcoholic extract against some five Pathogenic bacterial isolates, as Staphylococcus aureus that isolated from many infection by the test was accomplishby using wells diffusion method as well as measurement the optical density of bacteril growth of spectrophotometer, the results showed that increasing of average inhibition zone for(50 ,100 ,150 , 200)mg\ml alcoholic plant extract was non-significant between all concentration . while the bacterial growth for S.aureus be significant effected during Logartimic phase compare with control . The conclusion of the research was alcoholic Rosemary extract have impact inhibition growth Staphylococcal isolate , also we can recommend this plant extract replacement of common antibiotics.

Keyword: Rosmarins officinalis , Antibacterial , GC-mass , Staphylococcus aureus

1. Introduction

In recent years, the world has witnessed a great interest in medicinal plants, which are a natural source for treatment in the form of traditional preparations or effective compounds (Ghumima, 2018), as medicinal plants contain chemical compounds that have great importance and benefit as a result of their therapeutic activity and their functional effect on the organs of the human and animal body. (Youssef and Aliyah, 2019), so the researchers' attention was directed towards broader horizons by introducing chemicals taken from natural plant sources in the field of drug manufacturing and development, especially to control multi-resistant microorganisms to most types of traditional antibiotics, as plant extracts are a rich source of metabolites Secondary compounds that have a lethal activity against microorganisms, and among the most prominent compounds effective in killing bacteria (alkaloids, flavonoids, terpenoids, tannis, saponins, and phenols) (Omojate*et al.*, 2014).Among those medicinal plants, the rosemary plant, *Rosmarinus officinalis*, of the Lamiaceae family, stands out. It is one of the medicinal plants that has been widely used since ancient times and has a lot of uses due to its wide existence and ease of reproduction through roots or through cuttings, and it usually adapts to different environments in general (Al-Jubouri, 2014). It is grown in Central Asia, Southeast Africa, India, Australia, and southern Brazil (Yilan and Cristiane, 2010). It is also an important medicinal plant due to its vital efficacy, as it contains Rosmarinic acid and volatile oils by 1.5% (Amani *et al.*, 2017). Among the pathogenic bacterial species that are known to have a high ability to resist most antibiotics, *Staph.aureus* appears.

The infections that causd by *staphylococcus aureus* were this bacteria enter into life-threatening diseases unless treated as quickly as possible. *Staph.aureus* has been characterized by causing different types of diseases in several locations of the body. The virulence factors that characterize this type of bacteria have an important role in pathogenesis, as they cause a number of problems. health, starting from primary infections of moderate severity of low severity and reaching related diseases that require rapid intervention for treatment, including deep skin infections, endocarditis, chronic osteomyelitis, pneumonia and other diseases that lead to death (Greenwood *et al.*, 2012).

Aim of the study

Given the increase in the resistance of bacterial isolates of *staphylococcus aureus* to the antibiotics used and the difficulty of treating the diseases resulting from them, so the research aimed to know the role of the alcoholic plant extract of rosemary and its role in inhibiting this type of isolates and its impact on the beginning of the logarithmic phase of the bacteria.

2. Materials and methods

Rosmarins officinalis leaves were collected during the plant growth season, dried at room temperature, then ground using an electric mill. Extraction of the active substances was carried out using a soxhlet machine. Ethyl alcohol (ethanol) at a concentration of 70% was used as a solvent many concentrations were prepared (50, 100, 150, 200) mg/ml(Alsahlani,2015).

Gas chromatograph mass spectrometry

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The active compounds were identified in the alcoholic extract of rosemary plants using the Gas Chromatograph-Mass Spectrometry apparatus of the Ministry of Science and Technology - Iraq - Baghdad.

Bacterial isolates collection

All isolated were collected from patients with urinary tract infections and wounds, in addition to nasal swabs for patients attending hospitals in Najaf Governorate (Al-Sadr, Al-Hakim and Al-Najaf Teaching Hospitals). *Staph. aureus* was isolated and the isolates were subjected to microscopic and biochemical examinations to confirm them. A test was carried out with the Vitek compact 2 system to confirm the species of the bacteria by ID and AST card .

Bacterial susceptibility to antibiotics by Vitek 2 compact system

The diagnosis was made according to what was stated in (Koneman *et al.*, 2006), according to the instructions of the French company (BioMerieux), the device was used to confirm the diagnosis and test its sensitivity to antibiotics, through the use of a special diagnostic kit for the device, which is used to diagnose most types of Gram-positive bacteria. A sample in it needs two kits, one for diagnosing ID and another for antibiotic susceptibility testing called AST, as its diagnostic card contains (64) pits containing pits in dried medium and a color guide in which biochemical tests for the microorganism are conducted, according to the attached leaflet. The picture under experiment. A certain gap in the device, and the image device for the growth of bacteria by observing the turbidity.

1- The isolates were grown on mannitol salt agar media using the planning method and incubated at a temperature of (37) C for a period of (24) hours.

2- Put (5) ml of normal saline solution in the tubes of a spectrophotometer.

3- A carrier full of pure colonies was added to the tubes, then its optical density was compared with an intensity (2.80-3.80) using a spectrophotometer.

4- Transferring the cards to the filling door, so that it is transmitted through the card within a period of time (10-15) minutes, then the device works to cut the transport tube.

5- The cards were transferred, after the stuck inside it, to a room inside the device that represents the incubator, and then the device's work area, analyzing the results, and then reading them electronically within (6) hours.

Antimicrobial Activity

For the purpose of testing the effectiveness of the alcoholic extract against Staph. aureus, the method of diffusion in agars by wells as Mueller Hinton agar medium was used. The experiment was repeated three times and the average zones of inhibition were calculated(Zinedne and Faid, 2007). In addition to measuring the effect using a spectrophotometer, as part of the growing colonies was taken on the nutrient agar medium and planted in tubes containing (1) ml of BHI broth medium, then an equal volume of alcoholic extract of rosemary was added to the tubes, leaving a tube containing a bacterial culture without extract. The tubes were counted as a positive control, the tubes were incubated for different periods of time and with a time difference (2) hours (0, 2, 4, 6, 8 and 10) hours, then the optical density was measured using a spectrophotometer with a wavelength of (630) nanometers and the zero of the device on the medium BHI broth before reading(Alan and Hennessy, 2009).

3. Results and Discussion

The results shown in Table (1) the compounds that were identified and identified by gas chromatography-mass spectrum of the ethanolic extract of rosemary plant contained many active chemical compounds, and among these compounds, which occupied the largest area of the total identified compounds, is (Bicyclo [3.1. 1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)- and 11-Octadecenoic acid, methyl ester and cis-13-Octadecenoic acid

Table (1) Some compound ethanolic rosmary extract that characterized by GC-mass.

Area%	detention time	Molecular weight g/mol	Molecular formula	compound name
21.88	8.970	150.22	$C_{10}H_{14}O$	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-
10.03	22.170	269.5	$C_{19}H_{36}O_2$	11-Octadecenoic acid, methyl ester
8.29	22.750	282.5	$C_{18}H_{34}O_2$	cis-13-Octadecenoic acid

The results shown in Figure (1) showed the results of testing the sensitivity and resistance of bacterial isolates to antibiotics, that all isolates were resistant to the antibiotics Benzylpenicillin, Oxacillin, Gentamicin, and Fusidic acid with a percentage of (100)%. This antibiotic inhibits protein synthesis by binding to the elongation factor EF -G-GDP, which participates in the protein translation process and thus inhibits the process of peptide transfer and disassembly of the ribosome. *Staphylococcus* bacteria resist this antibody when a mutation occurs in the *Fus* A gene, and some mutations can lead to a high level of resistance to antibiotics (Fernandes, 2016).

Staphylococcus aureus bacteria resisted Ciprofloxacin, Moxifloxacin, and Erythromycin by (80%). As for the antibiotics Teicoplanin and Vancomycin, the resistance rate to them reached (40%). These antibiotics convert Topoismerase and Gyrase into toxic enzymes that destroy the bacterial chromosome and generate Resistance to these antibiotics occurs when a specific genetic mutation occurs that weakens the interaction between antibiotics and the bacterial enzymes Topoismerase

and Gyrase (Katie *et al.*, 2014), while the percentage of resistance to Clidanycin and Rifampicin reached (60)%, and the isolates were resistant to Linezolid by (10)%. As for the antibiotic Tigecyclin, all isolates were sensitive to it.

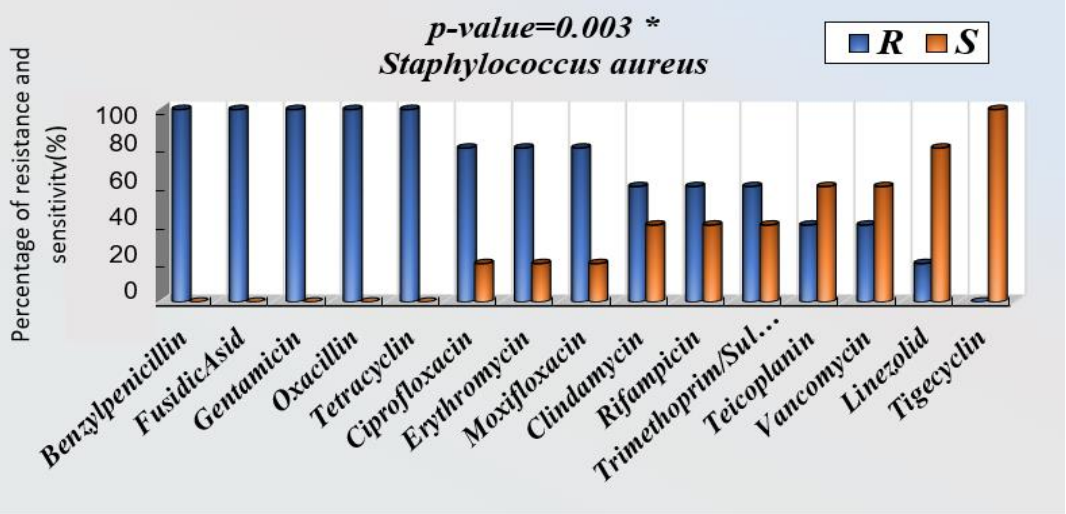

Figure (1) The percentage of resistance and sensitivity of Staphylococcus aureus isolates to the antibiotics used in the AST test

The results shown in Table (2) showed that the results of the statistical analysis that there were non-significant differences between the concentrations used and the isolates, and the results also showed that the concentration (200) mg / ml was the most effective on the bacterial isolates of the rate of diameters of inhibition, while the concentration was (50). (mg / ml) was the least effective on the studied isolates. The results of the study agreed with the findings of Jawad et al., (2020) in his study on rosemary leaves, that its extracts have effectiveness against microorganisms, and the inhibitory activity of rosemary is attributed to the chemical content of the active substances. It contains tannins, alkaloids, phenols, resins, and saponins, in addition to glycosides, as it is consistent with the findings of Hamza e tal., (2017), which demonstrated that rosemary extracts had an inhibitory effect on gram-positive and gram-negative bacteria.

The results shown in Figure (2, 3 and 4) also showed the effect of the alcoholic extract of rosemary on the growth of the bacterial isolates under study and compared them with the control coefficient, which showed a significant increase in the amount of growth in the time periods (0, 2, 4, 6, 8, 10) hours and that The amount of bacterial growth is directly proportional to the passage of time, while a difference was observed in its growth in the presence of the extract, as the curves show the extent of the bacteria's slowness in growth and the effect of the plant extract on it, and the alcoholic extract was efficient and active in inhibiting bacterial growth, it is known that the bacteria pass through four stages Sequential growth, which is the lag phase, the growth phase, the log (logaritmic) phase, the stationary phase and the death phase Surrounding it without dividing, and after that it enters the second phase, Log (Logaritmic) phase, in which the bacteria divide rapidly and multiply in number at a constant rate. In a late stage of this phase, nutrients decrease. Metabolic waste accumulates, and here the stationary phase begins, as cell division is slow until the bacteria die in the final death phase due to depletion of nutrients (Fujikawa and Morozumi, 2005).

The results of the current study showed that the plant extracts prevented the growth of bacteria in the first and second phases, especially in the logarithmic phase, and it is possible that the chemical compounds that make up rosemary have an effective role in inhibiting the growth of bacteria, such as phenolic compounds that change the nature of cellular protein and increase the permeability of the bacterial biofilm (Feeny, 1998), as well as the synergistic effect of other compounds such as groups of alkaloids, resins and tannins that make plant extracts effective against microorganisms (Draughon, 2004), as microorganisms can be eliminated or their growth is prevented by preventing the formation of the cell wall or the occurrence of Malfunctioning of proteins and nucleic acids, preventing their manufacture, or affecting the permeability of biofilms (Montville and Mattews, 2005).

Table (2) Inhibitory effectiveness of alcoholic extract of rosemary plants against Staph. aureus

bacterial isolation	The concentrations used for the alcoholic extract			
	50	100	150	200
Staph. aureus 1	9.33±0.58	13.67±0.58	14.67±0.58	15.33±0.58
Staph. aureus2	8.33±0.58	13.65±0.56	15.33±0.58	17.00±1.00
Staph. aureus3	12.67±0.58	14.00±1.00	15.67±0.58	16.00±1.00
Staph. aureus4	12.67±0.58	13.67±0.58	14.67±0.58	16.33±0.58
Staph.aureus5	13.67±0.58	14.67±0.58	15.67±0.58	16.33±0.58
L.S.D=1.28 p-value≤ 0.005				

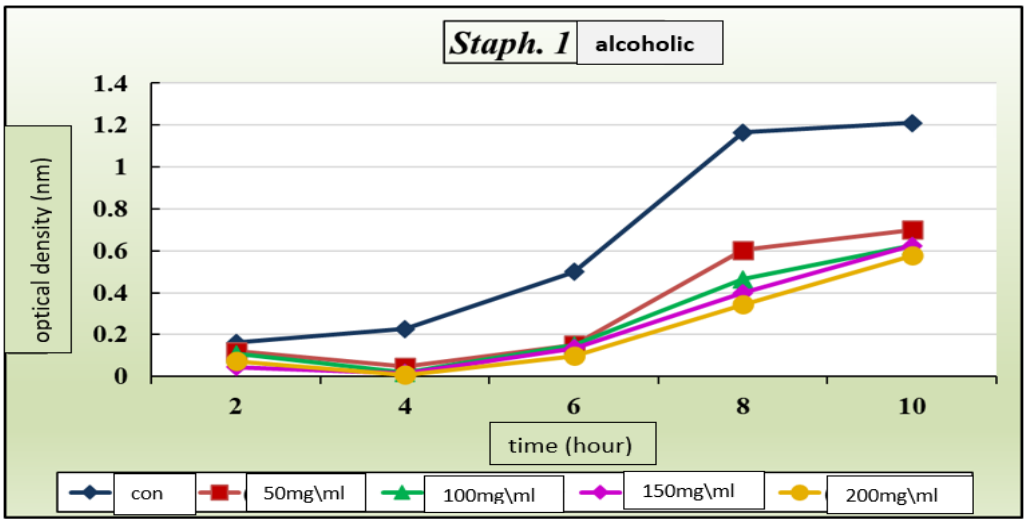

Figure (2): The effect of alcoholic extract of rosemary and the duration of incubation on the bacterial growth of Staph. aureus

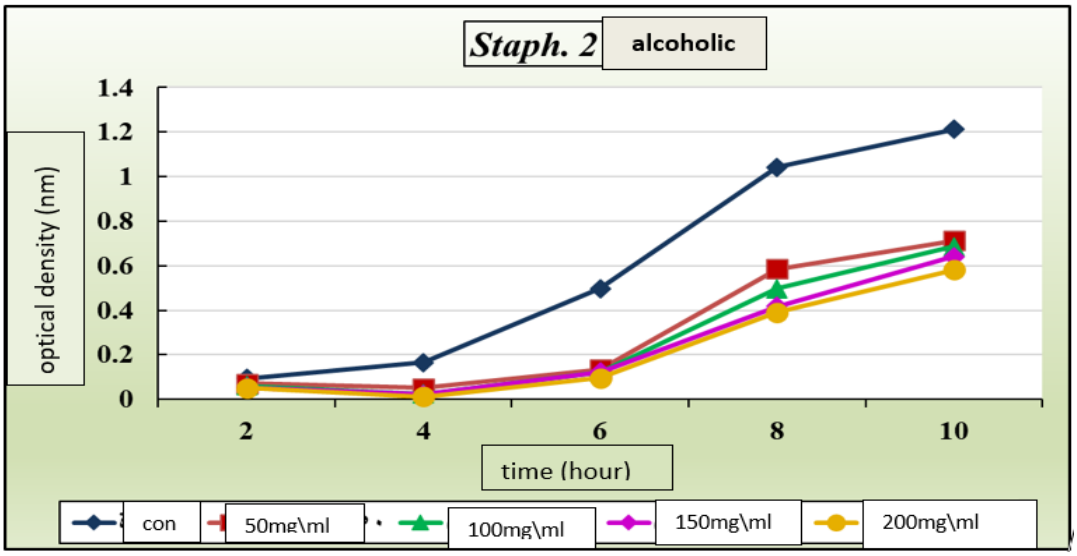

Figure (3): The effect of alcoholic extract of rosemary and the duration of incubation on the bacterial growth of taph. aureus

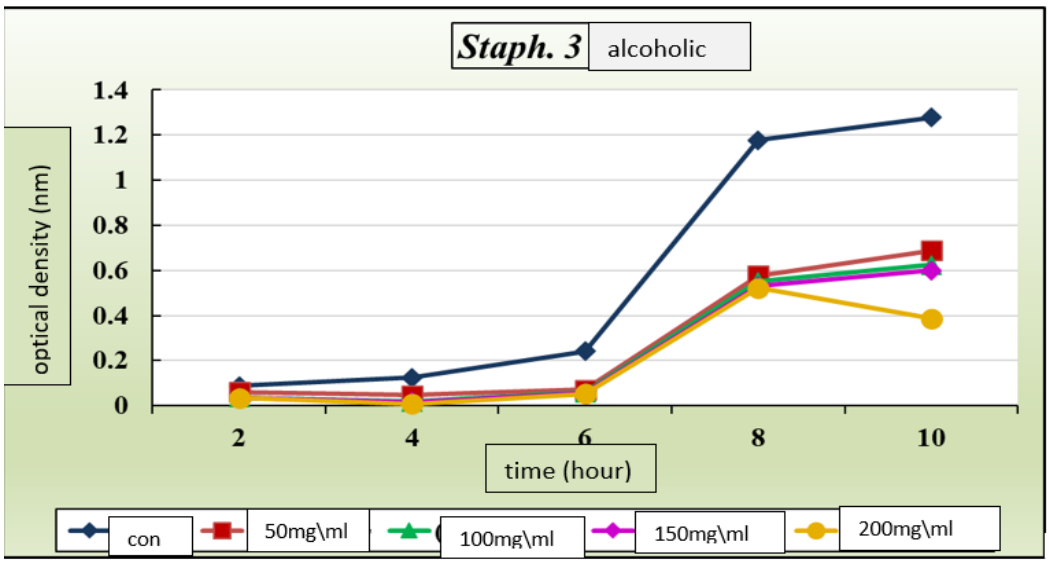

Figure (4): The effect of alcoholic extract of rosemary and the duration of incubation on the bacterial growth of Staph. aureus

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e conclude from the current study that the increase

in the inhibition diameter increases with the increase in the concentration of the extract, and it is possible to use the alcoholic extract as an alternative to the antibiotic to inhibit the growth of pathogenic bacteria.. The results depend on the method of extraction, sample collection and laboratory conditions, as researchers may obtain different results for the same plant used as a result of different methods of collection and extraction.

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