https://doi.org/10.48047/AFJBS.6.16.2024.2417-2423

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Synergistic effect of a mixture of rosemary plant extract with some antibiotics against some pathogenic bacterial species

ATMANI Abdelaali [1*] , Gouidoum Yacine Abd Elnour [2], Benchohra Mohamed [3]

[1]Department of Chimie, Faculty of Material Sciences, Ibn Khaldoun University, Zaaroura Road, 14000 Tiaret, Algeria

Email: atmaniabdelaali@hotmail.fr

[2]Department of Chimie, Faculty of Material Sciences, Ibn Khaldoun University, Zaaroura Road, 14000 Tiaret, Algeria

Email: yacinguid14@gmail.com

[3]Department of Chimie, Faculty of Material Sciences, belhadj bouchaib University, ain témouchent, Algeria

Email: madchimie@gmail.com

Article History

Volume 6, Issue 16, 2024

Received : 17 Aug 2024

Accepted : 12 Nov 2024

Published :21 Dec 2024

doi:10.48047/AFJBS.6.16.2024.2417-2423

Abstract

The purpose of this study is to investigate and evaluate the antibacterial activities of Rosmarinusofficinalis extracts (ethanol/water) against four strains of bacteria: Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus (ATCC 25923) and Bacillus Cereus (ATCC 11778) using the diffusion method.

The results revealed that all extracts have a certain biological activity against gram-negative and Gram-positive bacteria at 1000 and 5000 g/ml. In addition, the extracts (ethanol/water) showed the highest activity, is against Pseudomonas aeruginosa.

The combination of Rosmarinusofficinalis with each of the standard antibiotics: (SP25 µg) (Spiramycin), (MT5µg) (Metromidazole), AMX (amoxicillin), CZN (cefazolin), cxn (Cefalexin). They were more active and showed significant synergistic effects, extracts (ethanol/water) of medicinal plants Rosmarinusofficinalis, It also showed strong synergistic effects. The results of this study suggest that Rosmarinusofficinalis could be used to treat diseases caused by the organisms studied. Further, pharmacological and chemical studies can be carried out in order to isolate and identify the chemical constituents responsible for antimicrobial activity in selected plants.

Keywords: Rosmarinusofficinalis, medicinal plant, biological study, extract, antibiotic.

1-Introduction

Most bacterial infections are treated with antibiotics, but at present time the natural herbal treatments (folk medicine) has spread dramatically and sometimes without resorting to drugs and synthetic materials. However, due to the appearance of new strains of the bacteria and the weakness of chemotherapeutics and antibiotic resistance exhibited by pathogens has led to the screening of several medicinal plants for their potential antimicrobial activity [1-2]. An increasing number of reports dealing with the assessment of antimicrobial effects of different extracts of various medicinal plants are frequently available [3].

In this study, we are trying to measure the biological activity of rosemary and mixing it with some antibiotics.

Rosmarinusofficinalis , Rosemary is a perennial shrub that usually reaches about 01 meter (3.3 ft) in height. The linear leaves are about 01 cm (0.4 in) long and look somewhat like small, curved pine needles. It is dark green and shiny above, with a white underside and arched leaf edges. The small bluish flowers are borne in axillary clusters. Rosemary is quite resistant to
most plant pests and diseases. Plants grow easily from cuttings [5].

Figure 1: Fresh rosemary picked from the Radar area west of Tiaret, Algeria.

Rosemary is native to the Mediterranean basin. It is found mainly in arid and sunny areas, and does not like excessive drought [4]. Plant studies have unanimously agreed that the aerial parts of the rosemary plant are the parts used in traditional treatment (by herbalists), as well as in modern studies, especially the leaves[6]. Antidiarrheal, menstrual, wounds and scabies. Rosemary contains phenolic acids, the most important of which are rosmarinic acid, caffeic acid, and some flavonoids epinephrine, dicosamine, oxetine, ginquin, and hispidyline [7].

2-MATERIALS AND METHODS

Fresh Rosemary plant were collected from the mountains of Tiaret, western Algeria. The sample was deposited in the Scientific Research Laboratory at the Faculty of Materials Sciences, Ibn Khaldoun University - Tiaret, Algeria. The fresh plant is washed under running tap water, air dried under the dark, then homogenized into a fine powder and stored in a sealed container away from light and moisture.

Figure 2: Dried rosemary extracted from Radar w Tiaret, Algeria.

Plant sample extraction:

The extract was prepared by soaking 200 g of plant powder in a mixture of evaporated EtOH/H_2O (70/30) under low pressure. The resulting extract was diluted with distilled water and left overnight. The resulting residue was stored at 4 °C.

Figure 3: Extract (ethanol/water) of rosemary

Microorganisms All bacterial standard strains:

Escherichia coli (ATCC25922), Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus ATCC 25923andBacillus Cereus ATCC 11778) were obtained and diagnosed in the Microbiology Laboratory, Tiaret Hospital, Algeria.

Preparation of the bacterial culture media:

3.7 g of Muller Hilton agar were mixed with hot distilled water and autoclaved at 121°C and 02 atm for 15 min. After autoclaving, it was allowed to cool to 45°C in a water bath. Then the medium was poured into sterilized petri dishes with a uniform depth of approximately 05 mm [8].

Preparation of plant extract impregnated discs:

Whatman N°01 filter paper was used to prepare discs of 06 mm in diameter. They were sterilized by autoclaving and then dried during the autoclaving cycle. The discs were then impregnated with extract of the plants [9].

Disc diffusion method:

Disk propagation method:

The disc diffusion method for antimicrobial susceptibility testing was performed according to the standard method by Kirby-Bauer to evaluate the presence of antibacterial activities of the plant extract. A bacterial suspension adjusted to 0.5 McFarland standard (1.5 x 108 CFU/ml) was used to evenly inoculate Mueller Hinton agar plates using a sterile swab[10]. Discs impregnated with the plant extract were placed individually on the surface of Mueller-Hinton agar. The discs were spaced far enough apart to avoid reflection waves from the edges of the Petri dishes and overlapping damping rings. The plate was then incubated at 37°C for 18 h in an upside-down position to look for zones of inhibition. The zones of contraindications produced by susceptible organisms are defined by a circular area of clearing around the discs impregnated with the plant extract. The diameter of the zone of inhibition through the center of the disc was measured to the nearest millimeter.
The resulting residue of the extract stored at 04°C was tested at a concentration of 10–03 g/mL and prepared in DMSO[11].

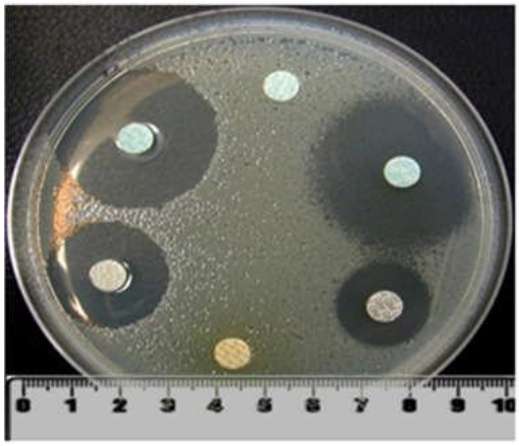

Figure 4: Measurement of the diameter of the inhibition zone in mm.

3-RESULTS

The results are summarized in the following table:

Table 1:Comparison of rates of inhibition of rosemary plants and their mixtures with antibiotics (in mm) against bacteria.

Diameter of inhibition zone (mm)				
Bacteria Strains / Plant extracts	Escherichia coli (ATTC 25922)	Pseudomonas aeruginosa (ATTC 27853)	Staphylococcus aureus (ATTC 25923)	Bacillus Cereus (ATCC 11778)
Rosemary (R_1)	18	22	19	11
$MT_{5\mu g}$ (R_2)	09	-	-	11
$MT_{5\mu g}$/Rosemary (R_3)	15	19	14	13
$\Delta R_1 = R_3 - R$ (1 ou 2)	**-03**	**-03**	**-05**	**+02**
$SP_{25\ \mu g}$(R_4)	13	-	12	-
$SP_{25\mu g}$/Rosemary (R_5)	25	20	24	20
$\Delta R_2 = R_5 - R$ (1 ou 4)	**+12**	**-02**	**+05**	**+09**
$CZN_{30\mu g}$(R_6)	29	-	26	-

$CZN_{30\mu g}$/Rosemary (R_7)	29	07	15	09
$\Delta R_3 = R_7 - R$ (1 ou 6)	**00**	**-15**	**-04**	**-02**
$CXN_{30\mu g}$ (R_8)	24	-	27	-
$CXN_{30\mu g}$ /Rosemary (R_9)	20	15	15	19
$\Delta R_4 = R_9 - R$ (1 ou 8)	**+02**	**-07**	**-12**	**+08**
$AMX_{25\mu g}$(R_{10})	11	-	30	08
$AMX_{25\mu g}$/Rosemary (R_{11})	34	28	25	30
$\Delta R_5 = R_{11} - R$ (1 ou 10)	**+16**	**+06**	**-05**	**+19**

DISCUSSION

The table (1) showed that:
A comparison between Rosmarinusofficinalis extract, antibiotics against bacteria, and their combination.
The Microbial growth inhibition of a Rosmarinusofficinalis extract tested in vitro by agar disk against 04 bacterial species showed significant bacterial activity against all bacteria tested (Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus Cereus).
Rosmarinusofficinalis and certain elements of the extraction of Rosmarinusofficinalis on the effectiveness of antibiotics in the mixture.
-R (extr / antibio) = R (extr): the combination (antibiotic/extract) does not cause synergy (neuter).
-R (extr / antibio) = R (antibio): the combination (antibiotic/extract) does not cause synergy (neuter).
-R (extr / antibio) < R (antibio) or R (extr): the combination (antibiotic/extract) does not cause synergy (inhibition).
-R (extr / antibio) > R (antibio) and R (extr): The mixture or the combination or the mix (antibiotic/extract) for a synergistic effect against the bacteria[12].
In the mixture (Rosmarinus/(CXN) it was more effective than the extract of Rosmarinus and the antibiotic (CXN) on Escherichia coli, Bacillus Cereuspar against the combination of extract of (Rosmarinus/CXN) is not effective as Rosemary extract and (CXN) antibiotic on the bacteria Pseudomonas aeruginosa, Staphylococcus aureus.

While the combination of Rosmarinus extract / (AMX), was more effective than Rosmarinus extract and antibiotic (AMX) on Escherichia coli, Pseudomonas aeruginosa and Bacillus Cereu and the opposite on Staphylococcus aureus bacteria although the combination of the extract of Rosemary (SP) was more effective than Rosemary extract and the antibiotic (SP) on Escherichia coli, Staphylococcus aureus and Bacillus Cereu and on the other hand on other bacteria while the combination of Rosmarinus extract / (CZN) has no synergistic (neutral) effect on Escherichia coli and for other bacteria was not as effective as Rosemary extract and antibiotic (CZN) on the bacteria Pseudomonas aeruginosa, Staphylococcus aureuset Bacillus Cereu and also as the combination of Rosmarinus extract / (MT) was not effective than Rosmarinus extract and antibiotic (MT) on the bacteria Pseudomonas aeruginosa and Bacillus Cereu, Staphylococcus aureus on the other hand on other bacteria.

The maximum antibacterial achieved by($MT_{5\mu g}$/Rosemary)is against Pseudomonas aeruginosaby rate 19mm.
The maximum antibacterial achieved by($SP_{25\mu g}$/Rosemary)is against Escherichia coli by rate 25 mm.
The maximum antibacterial achieved by ($CZN_{30\mu g}$/ Rosemary)is against Escherichia coli by rate 29 mm.
The maximum antibacterial achieved by ($CXN_{30\mu g}$ /Rosemary)is against Escherichia coli by rate 20 mm.
The maximum antibacterial achieved by ($AMX_{25\mu g}$/Rosemary)is against Escherichia coli by rate 34 mm.

CONCLUSION

Thanks to this work, we were able to study the plant Rosmarinusofficinalis and compare the antibacterial activity and the synergistic action of the extract of the flowers of the Rosmarinusofficinalis plant with certain antibiotics, as well as the extract (ethanol/water) against four bacterial isolates. We found that the extract of the plant (ethanol/water) had a significant effect on epidemiological bacterial groups, such as The bacteria Pseudomonas aeruginosa causes many different infections. Otitis externa, which is a benign condition that can occur in perfectly healthy individuals. It is caused by the penetration of water contaminated by bacteria into the ear while swimming in a pool. This condition is responsible for pain, itching and sometimes discharge, which means that this plant extract can be used to inhibit or suppress the spread of bacteria to ensure health protection.
We also discovered that the leaves of the Rosmarinusofficinalis plant had a synergistic effect with the extract (ethanol/water) as well as a synergistic activity with the antibacterials: Spiramycin (SP100 µg), Metromidazole (MT5µg), Amoxicillin (AMX23 µg),Cefazolin (CZN30 µg) and Cefalexin (CXN30 µg).

In the future, we want to separate active ingredients that have true synergy with each other and are responsible for suppressing pathogenic bacteria.
Finally, we hope to have achieved our research goal on the antibacterial activity and synergistic effects of an important plant. Or, at the very least, we have raised some doubts about an essential component of pharmacy at this time, so that we and others can work harder to improve the balance of scientific research in Algeria

ACKNOWLEDGMENT

The authors are thankful to Dr. OuassilaMokhtari, Aris Hospital, Batna ; Dr. A. Khalil, Hakim Saadan Hospital, Biskra; Dr. Ounissi and Mr. Berrah, Mohammed Bouthiaf Hospital, Ouargla 30000, Algeria for their assistance and providing the necessary facilities to carry out this work.

REFERENCES

[1]Benkeblia N, Antimicrobial activity of essential oil extracts of various onions (allium cepa) and garlic (Allium stivum). Lebensm-Wiss u-Technol2004 ; 37: 263-267.

[2]Jean-Claude Rameau et al., French forest flora: Mediterranean region, 2008.

[3]Preliminary phytochemical analysis and a comparative study of the antibacterial activity of Cyndondactylon (L) Pers roots.Research Journal of Pharmaceutical, Biological and Chemical Sciences,March–April 2016.

[4]Babamer ZY, Sekhri L, Al-Jaber H I, Al-Qudah MA, Abu Zarga, MH. Journal of Asian Natural Products Research 2012; 1-7.

[5] Britannica.com/plant/rosemary.

[6] G. Bedoux, C. Manguy, Biological Activities of the Essential from Selected Aromatic.

Plantes , Proceeding of the International Conference Bioatlas 2010 Transilvania.University of Brasov, Romania.

[7]Qudama, Ahmed. Nutritional dictionary and herbal medicine. Fifth Edition, Dar Al-Nafais Publications, Beirut.

[8] Cappuccino JG, Sherman N. Micro-A Laboratory manual. Addison Wesley Longman Inc 1999; 254-256.

[9] Swarnamoni D, Mukundam B, Shagufa A. Asian PharmClin Res 2013; 6 (4) : 136-139.

[10] A Comparative Study of the Antibacterial Activity ofCyndondactylon (L) pers ; its Synergic Effect withSome of the Standard Antimicrobsand Extracts of Some Medicinal Plants;Biomedical & Pharmacology Journal, 10, 2016.

[11]A comparative study of the antibacterial activity ofaristidapungensdesfleaves; its synergic effect with some of standard antimicrobsInternational Journal of Current Researchpp.39672-39680, October, 2016.

[12]Comparative study of inhibitory activity of extracts of some medicinal plants towards some pathogenic bacterial species History of Medicine2023 ,9(2): 632–636.