

EFFECT OF ROSMARINUS OFFICINALIS EXTRACT ON PATHOGENIC BACTERIA ISOLATED FROM RESPIRATORY TRACT INFECTION

Sura S Talib¹, Aljeboury, G.H.², Rawaa Adnan Khalaf³, Sara jawad kadhem⁴

^{1,2,3,4}Biotechnology Research Center/ AL-Nahrain university, iraq

ARTICLE INFO

Received: 25 June 2024

Revised: 15 July 2024

Accepted: 13 Augt 2024

Keywords:

Rosmarinus officinalis, RTI,
Extract, MIC, alcoholic, aqueous

Corresponding Author:

Sura S Talib

Email:

sura.s.t@nahrainuniv.edu.iq

Copyright © 2024 by author(s)

This work is licensed under the
Creative Commons Attribution
International License (CC BY 4.0).
[http://creativecommons.org/licenses/
by/4.0/](http://creativecommons.org/licenses/by/4.0/)



ABSTRACT

Nowadays, the prevalence of resistant bacteria is a result of the growing usage of antibiotics. The dearth of novel antimicrobial medications with less adverse effects than antibiotics is our problem. In conventional medicine, rosemary is a useful herb with numerous applications. In this research experiment, *Rosmarinus officinalis* was used to evaluate the antimicrobial effects on pathogens. The antibacterial properties of alcohol and aqueous extract were assessed using the well diffusion method on strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Strepto. agalctiae*, the minimum inhibitory concentration (MIC) of the extract on the growth of these bacteria was determined using the microplate method, and it was changed from 25 mg/ml to 100 mg/ml, these results imply that rosemary extract has an inhibiting impact on all strains, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Strepto. galactic*. Additionally, as the concentration was increased, the extract's effect increased.

INTRODUCTION

Advances in public health have focused on respiratory tract infections (RTIs) because of their wide distribution and the high rates of morbidity and mortality that have been documented globally (WHO, 2020). Infectious disorders that impact the respiratory system are known as RTIs (WHO, 2014). The host, the surrounding environment, and the causative agent interact to determine the severity of the disease. The clinical spectrum includes mild or asymptomatic infections as well as serious or deadly illnesses. Most often, these infections present as acute illnesses with a rapid onset of symptoms that may occur hours or days after the infection; these symptoms may include fever, coughing, sore throats, coryza, dyspnea, wheezing, and/or dyspnea (WHO, 2020). Since they contain elements with therapeutic potential, medicinal plants have been utilized for ages to treat human illnesses. Phytochemicals are becoming more and more popular as novel sources of natural antioxidant and antibacterial agents (Narayana et al., 2001). The aromatic and therapeutic qualities of rosemary (*Rosmarinus officinalis* L.) are highly prized. Evergreen and perennial, rosemary has

aromatic, needle-like leaves. It is a member of the family Lamiaceae. The biological bioactivities of rosemary extracts, including their hepatoprotective (Rańković et al., 2014), antifungal (da Silva Bomfim et al., 2020), insecticidal (Bajalan et al., 2017), antioxidant (Jiang et al., 2011), and antibacterial (Nieto et al., 2018) properties, have been documented in a number of investigations. In conventional medicine, it has been utilized as an analgesic, stimulant, and treatment for inflammatory conditions as well as for physical and mental exhaustion (Christopoulou et al., 2021). Carnosic acid and other phenolic acids, flavonoids, and terpenoids are the cause of rosemary extracts' antioxidant and antibacterial qualities (Ekambaram et al., 2016). Because of its potent antibacterial properties, *R. officinalis* is also frequently employed as a food preservative nowadays (Wang et al., 2012). Researchers are looking into the antibacterial activity of medicinal plants because microbial infections are becoming one of the biggest issues facing public health worldwide and because of the emergence of antibiotic resistance (Demissew & Dange, 2001). Aim of study: The purpose of the study was to find out whether *Rosmarinus officinalis* extract had antibacterial properties against pathogenic bacteria that were isolated from respiratory tract infections.

METHODOLOGY

Preparation of the herbal powder: The herbal plants (*Rosmarinus officinalis*) were collected from the local market of Baghdad. **Preparation of extraction:** Herbal powder (500 gram) was extracted in 1500 milliliters of either ethanol for alcoholic extraction and water for aqueous extraction for 12 hours by mixing on a magnetic stirrer, and filtered by whatman filter paper NO 1. Supernate was collected. Then the alcoholic extraction filtrates left to dry at 55 °C, herbal obtain from ethanol extract were mix with 1.5ml of DMSO, then filtered the mixture through Millipore filter 0.22 microfilter and stored as stock solution at 4°C until use while the aqueous extraction was then lyophilized to obtain a powdered formulation (Seyedemadia et al., 2016).

Investigation of Secondary Metabolites

Detection of Tannins tests

We added a few drops of 1% lead acetate solution to the extract. Tannins may have been present because a whitish or gelatinous precipitate formed.

Detection of polysaccharide

One milliliter of the extract and two milliliters of the Benedict reagent were combined, brought to a boil for five minutes, and then allowed to cool. Poly saccharides were present, as shown by the red deposit.

Detection of alkaloids (Dragendorff test)

Approximately dissolved Bismuth sub-nitrate in 0.2 milliliters of HCL (solution A). 600 mg of potassium iodide are present in 1 ml of distilled water in solution B. Alkaloids can be detected in the extract by adding the combined solution [A+B], which turns orange to brown.

Detection of Saponins

Shaking the extract solution well will be the next step in the detecting process. The extract's top layer of foam will show that saponins are present.

Detection of flavonoids

Alkaline reagent test: a brilliant yellow color indicates the presence of flavonoids when sodium hydroxide solution is combined with a little amount of extract solution and left.

Detection of polyphenolic compounds

The extract solution was mixed with a few drops of 3% ferric chloride solution to create a brown deposition.

Isolation and identification of bacteria: four samples of sputum were collected from patients with RTI (Respiratory tract infection) from Al-Yarmouk Hospital. All samples were cultured on blood and MacConkey agar, then Gram stain, Urease test, Catalase test and other media like mannitol salt agar, nutrient agar and milk agar was done for further isolation and identification of pathogenic bacteria.

Antibiotic resistance: The disk diffusion method, often known as the Kirby-Bauer method, is a widely used microbiological technique for assessing antibiotic susceptibility. In this process, agar plates that have been inoculated with a standardized bacterial suspension are covered with disks that have been impregnated with antibiotics. The microorganism's susceptibility or resistance to the tested antimicrobial drugs is then ascertained by measuring and comparing the zones of inhibition that develop around the disks to interpretative criteria

Antibacterial activity of the ethanol and aqueous herbal extracts:

Disk diffusion method on Mueller -Hinton agar was used to search for antibacterial activity [14] (Winn et al., 2006). A sterile cork borer was used to create three wells on a Muller Hinton agar plate that had previously been covered with bacterial culture.

Three wells were used: one held alcoholic extract, one had aqueous extract, and the third held simply DMSO as a control. After 24 hours of 37°C incubation, the diameter of the inhibitory zones was measured on the plates.

Determination of minimal inhibitory concentration (MIC):

Resazurin-based microplate method

Determination of Minimum Inhibitory Concentration (MIC) MIC determined on sterile 96 well micro titer plates according to the Clinical and Laboratory Standards Institute (CLSI) Guidelines (CLSI, 2011).

1. Add 100 microliter from the broth medium to all the microliter plate well
2. Add 100 microliters of extract in a concentration known as mg/ml400 to the first well
3. take 100 microliters from the first well of the container on the previous additions and add them to the second well and also take 100 microliters from the second to the third well until we get to the last well, we take 100 microliters and we wasted it.
4. Adding 10 microns of activated bacteria at 24 hours after comparing it with McCafferland to all the previous well except the last two well of each row (one contains only broth and extract and second only broth for control).
5. Incubate in the incubator for 18-24 hours
6. Preparation of Resazurin dye with concentration 0.015%

By adding 0.015g in 100 D.W, add 20 Microtiterplate Solution to all wells without exception and incubate the micro titer plate again in the incubator for two hours the results will show Change of color from blue to purple or red was considered as a sign of bacterial growth (Sarker et al., 2007).

RESULTS AND DISCUSSION

Phenolic acids, flavonoids, and terpenoids particularly carnosic acid and rosmarinic acid are responsible for the activities of rosemary extracts (Ekambaram et al., 2016). Phenolic acids, triterpenes, and phenolic diterpenes are the main active components of rosemary extract. Phenolic acids have important therapeutic qualities such as antibacterial, anti-inflammatory, and antioxidant effects (Farkhondeh et al., 2019).

Sample	flavonoides	saponins	polyphenols	alkaloids	tannins	glycosides
Alcoholic rosemary	+	+++	+++	-ve	++	+
Sample	flavonoides	saponins	polyphenols	alkaloids	tannins	glycosides
Aqueous rosemary	+	++	+++	+	+++	++

Table 1. Investigation of secondary metabolites

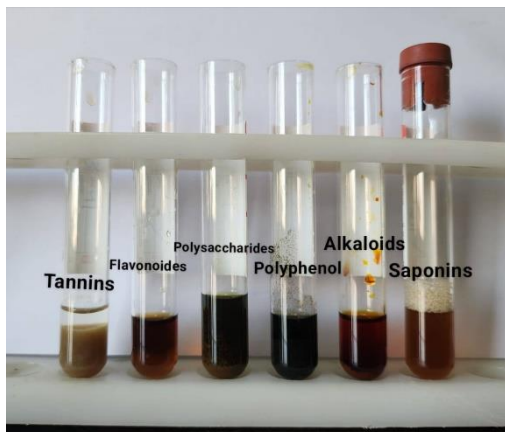


Figure 1: Aqueous rosemary



Figure 2: Alcoholic rosemary

Antibiotics sensitivity result

The susceptibility of many bacteria isolated from RTI cases, including *E. Coli*, *Streptococcus aureus*, *Pseudomonas aeruginosa*, and *Strepto. agalctiae*, was assessed using ten different types of antibiotics. 8 from 10 antibiotics have no effect on *Staphylococcus aureus*, *E. coli*, *pseudo. Aeruginosa* except Ceftriaxone, vancomycin has effect on *Staphylococcus aureus*, rifampin, amikacin effect on *E. coli* and levofloxacin, Ceftriaxone on *pseudo. Aeruginosa*, while 9 from 10 antibiotics has effect on *Strepto.agalctiae*, Antibiotics generally have little effect on bacterial isolates, these findings could be due to improper antibiotic use in Iraq, where it is common to obtain antibiotics without a doctor's approval, which has resulted in the development of strains that are highly resistant to various antibiotics, as show on table 2 below.

Table 2. antibiotics sensitivity result

antibiotics	Staph.aures	E. coli	Strepto.agalactia	Psedo.aeruginosa
DO	-	-	11mm	-
TE	-	-	15mm	-

DA	-	-	12mm	-
RA	-	11mm	-	-
CLR	-	-	12mm	-
LEV	-	-	20mm	19mm
AMK	-	11mm	15mm	-
CRO	13mm	-	14mm	11mm
VA	24mm	-	12mm	-
AM	-	-	12mm	-

Do: doxycycline, TE: tetracycline, DA: clindamycin, RA: rifampin, CLR: clarithromycin, LEV: levofloxacin, AMK: amikacin, CRO: ceftriaxone, VA: vancomycin, AM: ampicillin



Figure3: antibiotic sensitivity

Antibacterial activity of Rosemary extract

The antimicrobial impact of alcoholic and aqueous rosemary extract was detected using the same bacteria that were used to measure antibiotic sensitivity; the results showed a decrease in the isolate's growth rate as a result of the extract's action. Rosemary extract had bacteriostatic effect on Gram-positive bacteria and Gram-negative bacteria, utilizing the agar well diffusion method to observe the extract's antibacterial activity on harmful microorganisms revealed that the alcoholic extract has maximum zone of inhibition 20mm against *Pseudo. Aeruginosa*, and has minimum zone of inhibition 16mm against *staph. aureus* while aqueous extract has maximum zone of inhibition 19mm against *E. coli* and has minimum zone of inhibition 14mm against *staph. aureus*, alcoholic and aqueous extract they has minimum zone of inhibition against the same bacteria *staph. Aureus* and these bacteria have more antibiotic resistance in antibiotic sensitivity. It can be concluded that the kind of user solvent and extraction technique were key factors in determining the active component composition and the efficacy of alcoholic and aqueous extracts. The extracts' effectiveness may change after separation and purification, with the phenolic compounds in the first class and the alkaloids in the second class differing. Moreover, the extract's increased effectiveness may have an impact on the permeability of the cell membrane and the activity of the bacterial cell effect (Harborne, 1984).

Table 3. Antibacterial activity of Rosemary extract (inhibition zone in mm)

Bacteria	Control(dms0)	Alcoholic extract	Aqueous extract
<i>Staph. Aureus</i>	zero	16	14
<i>E.coli</i>	zero	17	19
<i>Strepto. agalctiae</i>	zero	18	16
<i>Pseudo. aeruginosa</i>	zero	20	15



Figure 4: Antibacterial activity of Rosemary extract

Minimum Inhibitory Concentration (MIC) of rosemary Extract

The MIC of alcoholic rosemary extract is between 25 to 100 mg/ml while the MIC of aqueous extract is between 50 to 100mg/ml as shown in figure 5, 6. Based on the findings of this research study, alcoholic extract of the test plant, possess antibacterial activity at low concentration while aqueous extract possesses antibacterial activity at high concentration, this is because water is not a good solvent for extraction also because alcoholic extract more phytochemicals that will inhibit growth of bacteria.

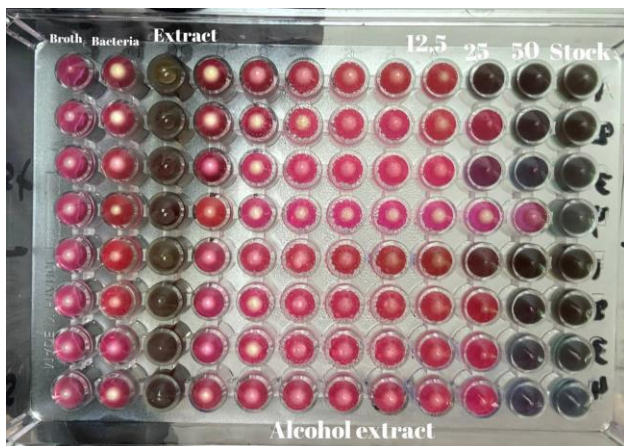


Figure 5: MIC of alcohol extract

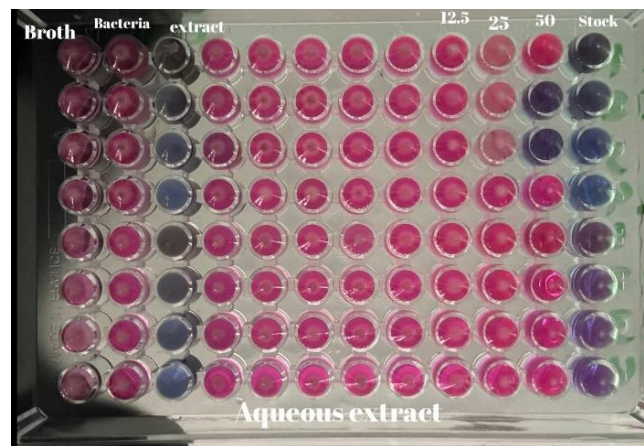


Figure 6: MIC of aqueous extract

The emergence of antibiotic resistance is one of the primary treatment issues that has received particular focus. Since rosemary extract has been shown to have antibacterial properties against a variety of bacteria, it is advised for the treatment of pathogen bacteria caused by infections. It is claimed that more study on medicinal plants could lead to potential substitutes for pharmaceutical medications that, although effective, have unfavorable side effects.

CONCLUSION

These results imply that rosemary extract has an inhibiting impact on all strains, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Strepto. agalactia*. Additionally, as the concentration was increased, the extract's effect increased.

REFERENCES

- World Health Organization. (2020). *WHO Severe Acute Respiratory Infections Treatment Centre: Practical Manual to Set Up and Manage a SARI Treatment Centre and a SARI Screening Facility in Health Care Facilities*. Geneva, Switzerland: World Health Organization.
- World Health Organization. (2014). *Infection Prevention and Control of Epidemic- and Pandemic-Prone Acute Respiratory Infections in Health Care: WHO Guidelines*. Geneva, Switzerland: World Health Organization.
- Narayana, K. R., Reddy, M. S., Chaluvadi, M. R., & Krishna, D. R. (2001). Bioflavonoid classification, pharmacological, biochemical effects and therapeutic potential. *Indian Journal of Pharmacology*, 33(1), 2-16.
- Rańković, A., Milanović, I., Pavlović, N., Ćebović, T., Vukmirović, S., & Mikov, M. (2014). Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) essential oil and its hepatoprotective potential. *BMC Complementary and Alternative Medicine*, 14, 225. <https://doi.org/10.1186/1472-6882-14-225>
- da Silva Bomfim, N., Kohiyama, C. Y., Nakasugi, L. P., Nerilo, S. B., Mossini, S. A. G., Romoli, J. C. Z., Graton Mikcha, J. M., Abreu Filho, B. A., & Machinski, M. Jr. (2020). Antifungal and antiaflatoxic activity of rosemary essential oil (*Rosmarinus officinalis* L.) against *Aspergillus flavus*. *Food Additives & Contaminants: Part A*, 37(1), 153-161. <https://doi.org/10.1080/19440049.2019.1678771>
- Bajalan, I., Rouzbahani, R., Pirbalouti, A. G., & Maggi, F. (2017). Antioxidant and antibacterial activities of the essential oils obtained from seven Iranian populations of *Rosmarinus officinalis*. *Industrial Crops and Products*, 107, 305-311. <https://doi.org/10.1016/j.indcrop.2017.05.063>
- Jiang, Y., Wu, N., Fu, Y. J., Wang, W., Luo, M., Zhao, C. J., Zu, Y. G., & Liu, X. L. (2011). Chemical composition and antimicrobial activity of the essential oil of rosemary. *Environmental Toxicology and Pharmacology*, 32(1), 63-68. <https://doi.org/10.1016/j.etap.2011.03.011>
- Nieto, G., Ros, G., & Castillo, J. (2018). Antioxidant and antimicrobial properties of rosemary (*Rosmarinus officinalis*, L.): A review. *Medicines*, 5(3), 98. <https://doi.org/10.3390/medicines5030098>
- Christopoulou, S. D., Androutopoulou, C., Hahalis, P., Kotsalou, C., Vantarakis, A., & Lamari, F. N. (2021). Rosemary extract and essential oil as drink ingredients: An evaluation of their chemical composition, genotoxicity, antimicrobial, antiviral, and antioxidant properties. *Foods*, 10(12), 3143. <https://doi.org/10.3390/foods10123143>
- Ekambaram, S. P., Perumal, S. S., Balakrishnan, A., Marappan, N., Gajendran, S. S., & Viswanathan, V. (2016). Antibacterial synergy between rosmarinic acid and antibiotics against methicillin-resistant *Staphylococcus aureus*. *Journal of Intercultural Ethnopharmacology*, 5(4), 358-363. <https://doi.org/10.5455/jice.20160906035020>
- Wang, W., Li, N., Luo, M., Zu, Y., & Efferth, T. (2012). Antibacterial activity and anticancer activity of *Rosmarinus officinalis* L. essential oil compared to that of its main components. *Molecules*, 17(3), 2704-2713. <https://doi.org/10.3390/molecules17032704>
- Demissew, S., & Dange, E. (2001). Basic and applied research on medicinal Ethiopia. In *Proceedings of the national workshop on conservation and sustainable use of medicinal plants in Ethiopia* (pp. 29). Addis Ababa, Ethiopia.

- Seyedemadia, P., Rahnema, M., & Bigdeli, M. R. (2016). The neuroprotective effect of rosemary (*Rosmarinus officinalis* L.) hydro-alcoholic extract on cerebral ischemic tolerance in experimental stroke. *Journal of Traditional and Complementary Medicine*, 6(4), 382-387. <https://doi.org/10.1016/j.jtcme.2015.12.001>
- Winn Jr., W., Allen, S., Janda, W., Koneman, E., Procop, G., Schreckenberger, P., & Woods, G. (2006). *Konemann's Color Atlas and Diagnostic Text of Microbiology* (6th ed.). Philadelphia, PA: Lippincott Williams & Wilkins.
- Clinical and Laboratory Standards Institute (CLSI). (2011). *Performance standards for antimicrobial susceptibility testing*. Wayne, PA: Clinical and Laboratory Standards Institute.
- Sarker, S. D., Nahar, L., & Kumarasamy, Y. (2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods*, 42(4), 321-324. <https://doi.org/10.1016/j.ymeth.2007.01.006>
- Farkhondeh, T., Samarghandian, S., & Pourbagher-Shahri, A. M. (2019). Hypolipidemic effects of *Rosmarinus officinalis* L. *Journal of Cellular Physiology*, 234(9), 14680-14688. <https://doi.org/10.1002/jcp.28361>
- Harborne, J. B. (1984). *Phytochemical methods: A guide to modern techniques of plant analysis* (2nd ed.). London, UK: Chapman and Hall.