



Evaluation of Antimicrobial Activity of *Rhazya Stricta* (Apocynaceae) Extract Prepared with Different Solvents on *Staphylococcus Aureus* (Staphylococcaceae) Isolated from Humans

Maryam Beigomi¹, Laleh shahraki-Mojahed², Batool Heydari-Sadegh³, Narjes Dahmardeh⁴, Reza Rouhani⁵, Fereshteh Javadian^{6*}

¹ Department of Food Sciences and Technology, Zahedan University of Medical Science, Zahedan, Iran

² Department of biochemistry, School of medicine, Zabol University of medical sciences, Zabol, Iran

³ Emergency medicine specialist, Zabol University of medical sciences, Zabol, Iran

⁴ Department of Anatomical sciences, Faculty of medicine, Zabol University of medical sciences, Zabol, Iran

⁵ Department of Neurosurgery, Amiralmomenin Hospital, Zabol University of medical sciences, Zabol, Iran

⁶ Zabol Medicinal Plant Research Center, Zabol University of Medical Sciences, Zabol, Iran

*Corresponding Author E-mail: Fereshteh.Javadian@yahoo.com

Received: 23 April 2021, Revised: 23 May 2021, Accepted: 06 June 2021

ABSTRACT

Introduction: Bacterial resistance to antibiotics, the first major concern in the 1960s, has reappeared worldwide over the past 20 years. Because these bacteria are not resistant to various conventional therapies, the medicinal and herbal plants used in different countries should be evaluated for their therapeutic potential. These valuable biological resources are a repository of complex active molecules. Therefore, in this study, we tried to evaluate the antimicrobial activity of some medicinal plant extracts on *Staphylococcus aureus* isolated from humans.

Methods: *Rhazya stricta* was collected and their species were identified in the botanical laboratory of University of Zabol. To prepare plant extract, 40 g of dried leaves were used in 400 cc of solvent (aqueous, ethanol, methanol, ethyl acetate, and hydro-alcoholic). The various strains of *Staphylococcus aureus* used in this study were isolated from the human nose and identified by biochemical, bacteriological and growth tests as well as standard tests. Antimicrobial effects were investigated by well diffusion method in Müller Hinton agar medium. Statistix ver10 software was used for statistical calculations. Mean comparison was performed using the LSD at the level of one percent and Excel software was also used to draw the shapes.

Results: *Rhazya stricta* extracts had different inhibitory zone diameters against

Staphylococcus aureus at 100 ppm dilution ($p < 0.01$). *R. stricta* ethanolic extract had the greatest effect (average 8.3 mm) on inhibiting the growth of 6 strains of *S. aureus*. Then aqueous and hydroalcoholic extracts with an average of 7 mm were in the next ranks. The ethyl acetate extract had the lowest effect on the inhibition of *S. aureus*. The lowest MIC of *R. stricta* ethanolic extract against *S. aureus* samples was 3.1 ppm, which was inhibited by four strains. The lowest MBC was 6.2 ppm.

Conclusion: Considering the side effects of chemical drugs and antibiotics as well as the potential effect of ethanolic extract of *Rhazya stricta* on *Staphylococcus aureus*, it is recommended to use ethanol solvent to evaluate the antimicrobial activity of *R. stricta*.

Keywords: d-tocopherol, Harmal, Tetrahydrosecamine, strictanol, genotoxic.

1. Introduction

Bacterial resistance to antibiotics, the first major concern in the 1960s, has resurfaced worldwide over the past 20 years. Therefore, World Health Organization (WHO) and other health organizations have declared war on human microbial pathogens, especially those acquired at the hospital, and have made drug discovery a top priority for these diseases. Because these bacteria are not resistant to various conventional therapies, medicinal and herbal plants used in different countries should be evaluated for their therapeutic potential. These valuable biological resources are a repository of complex active molecules [1].

Iran has a long history traditional medicine and the use of medicinal plants in the treatment of diseases. The need to pay attention to the science of using medicinal plants has increased for the following reasons: 1- The richness of plant flora in Iran and the high knowledge of Iranians in the use of medicinal plants, 2- Existence of reputable scientific centers in the cities of Isfahan, Shiraz, Rey, 3- Existence of authoritative scientific sources such as Bu-Ali Sina's law book, 4- Famous scientists such as Bu-Ali Sina and Razi who popularized medicine with medicinal plants among the people of Iran, and 5-Iranians' mutual interest in medicinal plants[1-4].

The study of medicinal plants to discover new methods of treatment, which have fewer side effects and higher economic value, has become particularly important worldwide [5-8]. According to published reports, more than 30% of herbal medicines are currently used in hospitals and clinics [9]. Antibiotics are valuable drugs for the treatment of many human diseases, however, overuse of these drugs will lead to microbial resistance. Therefore, scientists have prioritized research on different parts of medicinal plants to discover new drugs of plant origin [10-12].

Rhazya stricta belongs to the Apocynaceae family and the Rauwolfioideae subfamily. This species is distributed in Iran, Afghanistan, Pakistan, India, Iraq, Oman, Yemen, and Saudi Arabia (REF). *R. stricta* is known in Saudi Arabia as an Espand. *Rhazya stricta* is an evergreen and small plant. This plant has been used as a medicine to treat diseases in Afghanistan, India, Iran, Iraq, Pakistan, Qatar and Saudi Arabia [1, 13]. *R. stricta* (known in Saudi Arabia as harmful) is a poisonous, evergreen, small, erect shrub with hairless leaves [1]. *R. stricta*, also known as Harmal, is widely distributed in Saudi Arabia and South Asia. Harmal seed oil is considered as a potentially rich source of d-tocopherol, a major form of vitamin E [14].

R. stricta and its metabolites are used to treat diseases such as cancer, skin

diseases, hypertension, rheumatism, sore throat, syphilis and fever [15]. Various studies have shown that different parts of *R. stricta* contain many phytochemical elements such as alkaloids, flavonoids and terpenes [16, 17]. Research on *R. stricta*, a plant native to Asia, has shown that it has a number of healing properties. Antimicrobial activity of different concentrations of five solvent extracts, i.e. aqueous alkaloids, non-aqueous alkaloids, organic alkaloids, organic non-alkaloids and complete aqueous extracts, derived from *R. stricta* leaves were evaluated against several drug-resistant human and it has been concluded that organic alkaloid extract has been the most effective against *Escherichia coli* and MRSA [1].

Raw ethanolic extract of *R. stricta* fruit has antimicrobial activity [14]. Methanolic and chloroform root extracts of *R. stricta* plant have antimicrobial and antifungal activity against *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans* [18] and also *R. stricta* aqueous extract has shown antimicrobial activity against *S. aureus* [1].

Silver nanoparticles synthesized using *R. stricta* root extract in combination with xylitol have shown potential antimicrobial activity because they are single, dispersed, stable, and less aggregated. Antibacterial activity of *R. stricta* root extract is increased when combined with AgNP synthesis. Thus, synthesized AgNPs enhance the therapeutic effect, enhance *R. stricta* drug levels, and provide a strong source of an antimicrobial agent against gram-positive and gram-negative organisms [14].

Tetrahydrosecamine isolated from *R. stricta* has shown broad-spectrum antimicrobial activity and has been shown to be more active against *E. coli* and *Pseudomonas aeruginosa*. Raw ethanolic extract of *R. stricta* fruit has antibacterial, lipoxygenase and acetylcholinesterase activity. Chloroform and methanol extracts of *R. stricta* roots have shown antimicrobial and antifungal activity against *B. subtilis*, *P. aeruginosa*, *E. coli*, *S. aureus*, *Aspergillus terreus*, *Aspergillus flavus* and *C. albicans*. Aqueous extract of *R. stricta* leaves has genotoxic effects that have been shown by cytogenetic and molecular evaluation against fungal cells [1].

For thousands of years, humans have used natural ingredients as one of the most important sources for treating diseases. Plants have always played an important role in the treatment and health of human societies. Due to the fact that long-term use of antibiotics has led to the emergence of resistant bacteria and has caused significant clinical problems in the treatment of infectious diseases, the aim of this study was therefore to investigate the antimicrobial activity of various extracts of the medicinal plant *R. stricta*.

2. Materials and methods

2.1. Herbal materials

Rhazya stricta was collected from Saravan (Coordinates: 27 ° 22'15 " N 62 ° 20'03 " E) and their species were identified by plant systematic specialist in the botanical laboratory of University of Zabol (Fig. 1).



Figure 1. Appearance characteristics of leaves (A, B), Stem (B), and flowers (A, C) of *Rhazya stricta* plant (photo by Azar Davari)

2.2. Extract preparation method

The amount of 40 g of plant leaves, dried in the shade and in the vicinity of air, was milled (IKA company model A11 basic in Germany) and then it was used in 400 mL of different solvents (distilled water, 96% ethanol, hydroalcohol (70% alcohol and 30% water), Methanol, ethyl acetate). After that, they were shaken for 48 h at room temperature on a shaker (UniEquip SKIR-601 L Germany). After the desired time, the extracts were filtered, then the solvent was evaporated at a temperature of less than 40 °C by a rotary device (Pars Azma Company, model R002, Iran). The obtained extracts were weighed and then 100 mg of the extract powder was dissolved in 1 cc of DMSO solvent. The extracts were refrigerated at 4 °C until used in antimicrobial experiments [19].

2.3. Isolation of *Staphylococcus aureus* from the human nose

Most carriers of *Staphylococcus aureus* carry the bacterium in their airways and noses. For this reason, samples of nasal swabs were taken from 200 healthy people in Sistan region. Then, using biochemical methods, 10 strains of *S. aureus* were isolated from the samples and cultured on blood agar and nutrient agar media. The purity of the isolated

bacteria was confirmed by mannitol, catalase and hot staining tests [20].

2.4. Preparation of microbial suspension

Microbial suspension was prepared from all strains in Müller-Hinton broth culture medium by inoculating a 24-hour fresh culture for each series of experiments. To do this, one loop of each microbe was inoculated in 5 cc solution of the above culture medium for 24 hours. The incubator was exposed to 37 °C. The microbial suspension was compared and adjusted with McFarland 0.5 solution by adding 0.9% normal saline, then this microbial suspension was used for inoculation in Müller-Hinton agar medium [21].

2.5. Well diffusion testing

To perform the well diffusion test, a 24-hour culture was first prepared from the tested bacteria, which contained approximately 10⁶ bacteria per milliliter. They were then inoculated on Müller Hinton agar using sterile swabs. Sterile wells with a diameter of 5 mm were then created on Müller Hinton agar medium. These wells were inoculated with 50 µL of ash extract (*Rhazya stricta*). Each well contained 5 mg of the studied compounds. Petridishes were incubated at 37±2 °C for 48 h. After the incubation period, the diameter of the growth

inhibition zone formed around the tested wells was examined under light using a caliper measuring ruler. The millimeter face was reported for statistical work.

2.6. Determination of the MIC and MBC of the extract on *Staphylococcus aureus*

To determine the minimum inhibitory concentration (MIC) of plant extract used by eye method, the first 100 microliters of Mueller Hinton Broth (made by Merk-Germany) was added to each well of the titer plate [22, 23]. Then in the first well, 100 μ L of dilution of 20 mg/mL of essential oil was added and after mixing, 100 μ L of the first well was removed and added to the second well. Similarly, the construction of double dilutions in other wells continued. It should be noted that in this case, the first well contains 20 mg per microliter of essential oil, and thus in subsequent wells, this amount is reduced to half of the previous well. Finally, 10 μ L of each bacterial suspension (cfu = 10^8 1.5 per mL, half McFarland) was added to the wells. DMSO was added to the negative control well (without essential oil) and then the microtiter plate was incubated for 37 hours at 37 °C.

The minimum inhibitory concentration (MIC) was defined as the lowest concentration required for stopping the growth of bacteria at the end of 24 hours of incubation. To determine the minimum bactericidal concentration (MBC), 10 microliters of the wells were secondary cultured on a

nutrient agar medium (manufactured by Merk-Germany) at the end of 24 hours of incubation, and plates were examined for bacterial growth for 24 hours. The lowest concentration of essential oil in which 99.9% of bacteria did not grow was considered as MBC [22, 23]. All antimicrobial tests were repeated 3 times.

2.7. Data analysis

Statistix 10 software was used for statistical calculations. Mean comparison was performed using the least significant difference (LSD) at the level of one percent and Excel software was also used to draw the shapes.

3. Results

3.1. Diameter of inhibition zone of *Rhazya stricta* plant extracts on *Staphylococcus aureus*

The diameter of the inhibitory zone of plant extracts against *Staphylococcus aureus* at a dilution of 100 ppm was investigated and it was found that different extracts had different effects on inhibiting the growth of *S. aureus* ($p < 0.01$) (Fig. 2; Table 1). LSD test showed that ethanolic extract of *R. stricta* had the greatest effect (mean 8.3 mm) on inhibiting the growth of 6 strains of *S. aureus*. Then aqueous and hydroalcoholic extracts with an average of 7 mm were in the next ranks. The least effect on the inhibition of *S. aureus* was the ethyl acetate extract of *R. stricta* (Fig. 3).

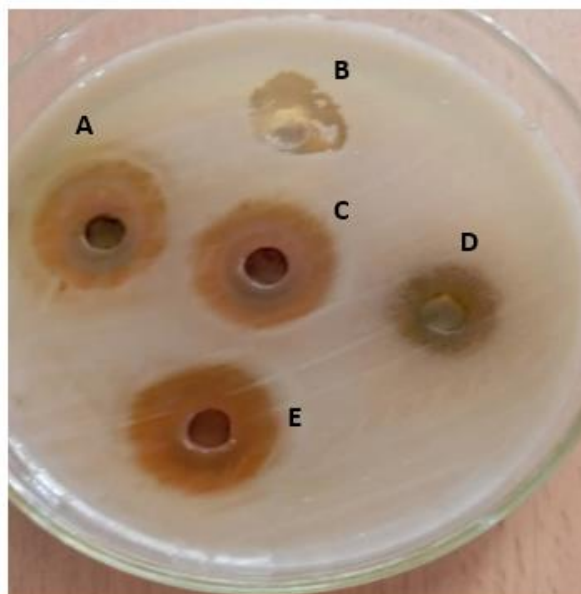


Figure 2. Inhibitory zone diameter results of 100 ppm concentration of Eshvarak extracts of methanol (A), ethyl acetate (B), hydro-alcohol (C), ethanol (D), and water (E).

Table 1. Analysis of variance of inhibitory zone diameter of plant extract against *Staphylococcus aureus* at dilution of 100 ppm.

Source	DF	MS (<i>Staphylococcus aureus</i> strain)									
		1	2	3	4	5	6	7	8	9	10
Plant extracts	4	18.9**	44.1*	9.6*	71.4*	24.9*	51.6*	34.5*	8.1*	18.9*	60*
Error	10	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	14										

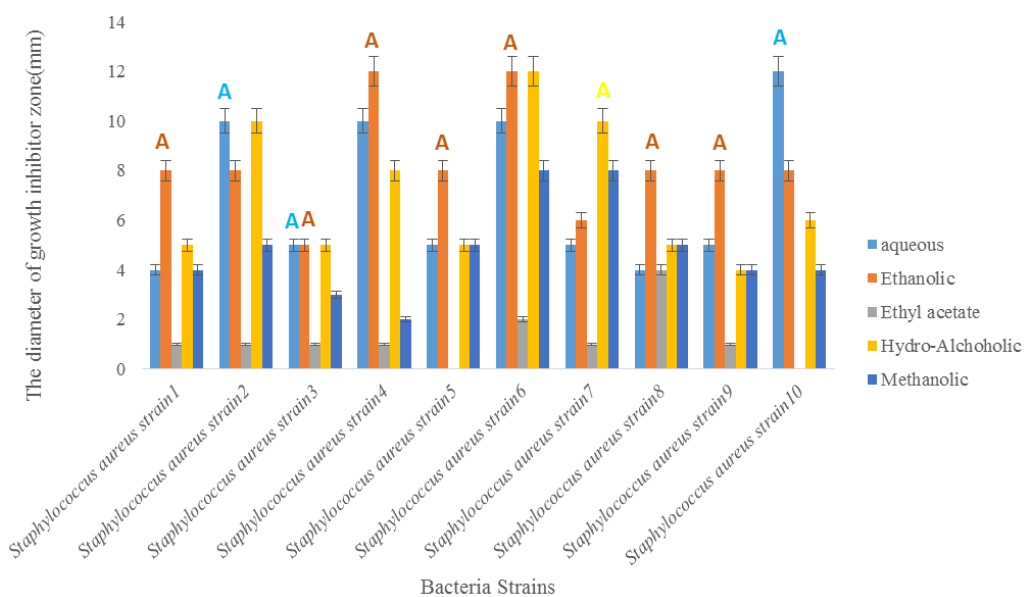


Figure 3. Inhibitory zone diameter of *Rhazya stricta* extract against *Staphylococcus aureus* at 100 ppm dilution. Similar letters indicate no significant difference

3.2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts on *Staphylococcus aureus*

The lowest MIC of aqueous extract of *Rhazya stricta* against samples of *Staphylococcus aureus* was 3.1 ppm, which inhibited only one strain. While the maximum MIC was 25 ppm. The lowest MBC was equal to 6.2 ppm while the highest MBC was equal to 50 ppm (Table 2).

The lowest MIC of *R. stricta* ethanolic extract against *S. aureus* samples was 3.1 ppm, in which four strains were inhibited. While the highest MIC was equal to 12.5 ppm. The lowest MBC was equal to 6.2 ppm while the highest MBC was equal to 25 ppm (Table 2).

The lowest MIC of *R. stricta* ethyl acetate extract against *S. aureus* samples was 25 ppm, while the highest MIC was 50 ppm. The lowest MBC was 50 ppm while the highest MBC was 100 ppm (Table 2).

The lowest MIC of *R. stricta* hydroalcoholic extract against *S. aureus* samples was 12.5 ppm, while the highest MIC was 25 ppm. The lowest MBC was 25 ppm while the highest MBC was 50 ppm (Table 2).

The lowest MIC of *R. stricta* methanolic extract against *S. aureus* samples was 12.5 ppm, while the highest MIC was 25 ppm. The lowest MBC was 25 ppm while the highest MBC was 50 ppm (Table 2).

Table 2. MIC and MBC of *Rhazya stricta* extract prepared from different solvents against *Staphylococcus aureus*

<i>Staphylococcus aureus</i> strain	MIC-MBC of Extracts				
	aqueous	Ethanolic	Ethyl acetate	Hydro-Alcoholic	Methanolic
1	12.5-25	12.5-25	25-50	12.5-25	12.5-25
2	25-50	12.5-25	25-50	25-50	25-50
3	25-50	3.1-6.2	50-100	25-50	25-50
4	12.5-25	3.1-6.2	50-100	25-50	25-50
5	25-50	3.1-6.2	25-50	25-50	25-50
6	25-50	3.1-6.2	50-100	25-50	25-50
7	25-50	12.5-25	50-100	25-50	25-50
8	6.25-12.5	12.5-25	25-50	12.5-25	25-50
9	25-50	25-50	50-100	12.5-25	12.5-25
10	3.1-6.2	25-50	50-100	12.5-25	12.5-25

4. Discussion

Rhazya stricta ethanolic extract had the greatest effect (average 8.3 mm) on inhibiting the growth of 6 strains of *Staphylococcus aureus*. Then aqueous and hydroalcoholic extracts with an average of 7 mm were in the next ranks. The lowest effect on the inhibition of *S. aureus* was the extract of ash ethyl acetate. The lowest MIC of *R. stricta* ethanolic extract against *S. aureus* samples was 3.1 ppm,

which four strains were inhibited. While the highest MIC was equal to 12.5 ppm. The lowest MBC was equal to 6.2 ppm while the highest MBC was equal to 25 ppm.

The antibacterial effect of *Vaccinium corymbosum* leaf extract on the standard strain of *Salmonella typhimurium* was investigated and they indicated that the diameter of the growth zone was 23 mm for a concentration of 10 mg per disk

[24]. Also, the antimicrobial properties of *V. oxycoccus* were investigated by well diffusion method against gram-negative bacteria such as *S. typhimurium* and it was concluded that the diameter of the growth inhibition zone from the concentration of 1 mg/mL of ethanolic extract was between 13 and 20 mm [25]. In the present study, the diameter of the growth inhibition zone obtained from the ethanolic extract of *R. stricta* against *S. aureus* was between 6 and 12 mm, which is weaker than *V. oxycoccus*. However, the diameter of the growth inhibition zone depends on the type of bacteria and even the method of use [19]. The MIC value for blueberry extract (*V. corymbosum*) against *Salmonella enteritidis* strains has been reported to be between 450 and 1200 mg/mL [26]. In the present study, this value was equal to 3.1 ppm against *Staphylococcus aureus*.

The antibacterial properties of methanolic and ethyl acetate extracts of 8 species of seaweed were investigated and it was concluded that methanolic extract was more effective than ethyl acetate [27]. The antimicrobial properties of ethanol, methanol and chloroform extracts were investigated and it was concluded that methanolic extract shows the best results for both gram-positive and gram-negative bacteria [28]. Other studies have reported that methanolic extract has the highest antimicrobial properties [19, 29]. The antimicrobial properties of methanol, ethyl acetate, acetone and hexane algae extracts were investigated and it was concluded that the Estonian extract had a greater antibacterial effect than other extracts [27]. In the present study, ethanolic extract had the greatest effect, indicating the dependence of antimicrobial properties on the type of solvent and plant species and even the type of microbe under study.

Methanolic extract of red alga (*Gracillaria folifera*) had no inhibitory

effect on *E. coli* growth [30]. In another study, the ethyl acetate extract of the alga (*G. folifera*) on *E. coli* showed a relatively good inhibitory effect at different concentrations. The reason for the difference in the results was the algae species, the extraction method and the seasons of the harvest year [31]. In the present study, it was found that different solvents have different effects on antibacterial properties

Ethyl acetate extract of red algae (*Gelidiella acerosa*) had no effect on the growth of *S. aureus* [32]. The highest antibacterial power of methanolic extracts of red algae (*Gelidiella attenatum*, *Gelidiella spinulosum* and *Hypneme muciformis*) was against *S. aureus* [33] while in another study no inhibitory activity was observed on *S. aureus* bacterium in this regard, bacterial resistance and the metabolic activity of algae and algal species can be considered effective. Variation in antibacterial activity was also attributed to the extraction, solvent, and season in which the samples were collected [32]. In the present study, *R. stricta* ethyl acetate extract had the least effect on inhibiting the growth of *S. aureus*, which was similar to the presented research.

Proanthocyanidin in *V. vitisidaea* and *V. macrocarpon* have been reported to be a potent inhibitor against bacteria [34]. *V. angustifolium* contains compounds such as monomeric phenols, proanthocyanidins and anthocyanins, which have been effective against some pathogenic bacteria, including *S. typhimurium* [35]. *V. corymbosum* contains phenolic compounds such as chlorogenic acid, alllic acid, quercetin and quercetin-3 galactoside, which have been reported to be the main growth inhibitors of *Salmonella enteritidis* [26]. Ethyl acetate solvent has been shown to be effective against *S. aureus* due to the extraction of active compounds including hydroxyl fatty acids, glycolipids, steroids,

phenolics and terpenoids from the green alga *Enteromorpha prolifera* [36]. However, some of the active compounds extracted (including nitrogenous compounds) in algae by ethyl acetate solvent had little inhibitory activity on some pathogens [37]. In the present study, some solvents have more antibacterial properties, which indicate the ability of different solvents to extract active substances, so it is suggested to study plant extracts using different solvents to determine the most effective solvent and the most effective active substance.

Plant growth and yield in ecosystems are affected by various factors such as species type, region climate, soil environment, altitude and geographical location. Each of these factors can have a significant impact on the quantity and quality of plant products [38, 39]. The diverse climatic conditions of Iran have created suitable environments for the growth of 75,000 to 80,000 highly distributed medicinal plants [40]. In other words, these climatic differences have led to the creation of various ecotypes of different species of medicinal plants [13]. These plants are reservoirs rich in secondary metabolites and primary active ingredients. It has been found that most of the plant essential oils extracted from plants have insecticidal, antifungal, antiparasitic, antibacterial, antiviral, antioxidant and cytotoxic properties [41]. This antioxidant activity of medicinal plants depends on the total amount of their phenolic and flavonoid compounds [42]. Determining the amount of effective compounds and antioxidant value in medicinal plants is of great importance due to their wide application in various industries such as food, pharmaceutical, cosmetic, industrial, etc. [43].

The relative superiority of the use of medicinal plants, herbal medicines and chemical drugs based on economic and

therapeutic criteria was examined and it was concluded that medicinal plants were preferred in terms of cheapness, but their availability to the public was much lower than that of chemical drugs. Also, the side effects of chemical drugs are much higher than those of herbs and herbal medicines. On the other hand, the shelf life of medicinal plants has a lower relative weight compared with chemical drugs. Finally, it has been suggested that it seems necessary to encourage the general public, knowing the economic and therapeutic benefits of the use of medicinal plants, in order to promote and develop the culture of consumption of this group of useful drugs [44].

5. Conclusion

Due to the very good values of MIC and the diameter of the growth inhibition zone obtained in the present study and other reported cases, it should be noted that oral consumption of *Rhazya stricta* will expose high doses of active ingredients in the gastrointestinal tract to *Staphylococcus aureus*. By preventing the growth and multiplication of this bacterium, it will prevent the onset of various infections in the human body. Therefore, evaluation and confirmation of anti-*Staphylococcus aureus* properties will be useful even in high doses. It is possible that some of the differences in the results of this study may be due to differences in the type of plant species, the method of extraction or the type of solvent used, when compared with other studies. However, the results of this study are consistent with those of other researchers in confirming the antimicrobial activity of *R. stricta*.

Conflict of interest

None of the authors have any conflict of interest to declare.

Consent for publications

All authors approved the final manuscript for publication.

Availability of data and material

Data are available on request from the authors.

Funding/Support

Not applicable

Ethics approval and consent to participate

No humans or animals were used in the present research.

References

- Khan R, Baeshen M N, Saini K S, Bora R S, Al-Hejin A M, Baeshen N A. (2016). Antibacterial activities of *Rhazya stricta* leaf extracts against multidrug-resistant human pathogens. *Biotechnology & Biotechnological Equipment*, 30(5): 1016-1025. <https://doi.org/10.1080/13102818.2016.1209087>
- Fazeli-Nasab B, Sayyed R Z, Sobhanizadeh A. (2021). In Silico Molecular Docking Analysis of α -Pinene: An Antioxidant and Anticancer Drug Obtained from *Myrtus communis*. *International Journal of Cancer Management*, 14(2): e89116. <https://doi.org/10.5812/ijcm.89116>
- Fazeli-Nasab B, Khajeh H, Rahmani A F. (2021). Effects of culture medium and plant hormones in organogenesis in olive (CV. Kroneiki). *J. Plant Bioinform. Biotech.*, 1(1): 1-13. <https://doi.org/10.22034/jpb.2021.122590>
- Fazeli-Nasab B. (2021). Biological Evaluation of Coronaviruses and the Study of Molecular Docking, Linalool, and Thymol as orf1ab Protein Inhibitors and the Role of SARS-CoV-2 Virus in Bioterrorism. [Research]. *journal of ilam university of medical sciences*, 28(6): 77-96. <https://doi.org/10.29252/sjimu.28.6.77>
- Davari A, Solouki M, Fazeli-Nasab B. (2018). Effects of jasmonic acid and titanium dioxide nanoparticles on process of changes of phytochemical and antioxidant in genotypes of *Satureja hortensis* L. *Eco-Phytochemical Journal of Medicinal Plants*, 5(4): 1-20. http://ecophytochemical.gorganiau.ac.ir/article_605985.html?lang=en
- Rasouli H, Farzaei M H, Khodarahmi R. (2017). Polyphenols and their benefits: A review. *International Journal of Food Properties*, 20(sup2): 1700-1741. <https://doi.org/10.1080/10942912.2017.1354017>
- Mehrabi A-A, Fazeli-Nasab B. (2012). In vitro culture of *Allium scorodoprasum* spp. Rotundum: callus induction, somatic embryogenesis and direct bulblet formation. *Intl. J. Agri. Crop Sci*, 4(1): 1-7.
- Fazeli-nasab B, Fooladvand Z. (2014). Classification and Evaluation of medicinal plant and medicinal properties of mastic. *International Journal of Advanced Biological and Biomedical Research*, 2(6): 2155-2161.
- Yang Y, Xi-Qiang L, Chun-Ping T. (2008). Natural products chemistry research 2006's progress in China. *Chin J Nat Med*, 6(1): 70-78. [https://doi.org/10.1016/S1875-5364\(09\)60008-X](https://doi.org/10.1016/S1875-5364(09)60008-X)
- Islam S, Rahman A, Sheikh M I, Rahman M, Jamal A H M, Alam F. (2010). In vitro antibacterial activity of methanol seed extract of *elettaria cardamomum* (L.) maton. *Agriculturae Conspectus Scientificus*, 75(3): 113-117.
- Rasouli H, Yarani R, Pociot F, Popović-Djordjević J. (2020). Anti-diabetic potential of plant alkaloids: Revisiting current findings and future perspectives. *Pharmacological*

- research, 155: 104723.
<https://doi.org/10.1016/j.phrs.2020.104723>
12. Fooladvand Z, Fazeli-nasab B. (2014). Antibacterial activities of *Stachys lavandulifolia* Vahl. extract against eight bacteria. *Journal of Herbal Drugs (An International Journal on Medicinal Herbs)*, 5(1): 13-18.
 13. Saeidi S, Fazeli-Nasab B. (2019). Evaluation of antibacterial and antifungal activity of various extracts of the *Rhazya stricta*, *Capparis spinosa*, *cretica* Cressa. *New Findings in Veterinary Microbiology*, 2(1): 57-66.
<https://doi.org/10.35066/j040.2019.454>
 14. Shehzad A, Qureshi M, Jabeen S, Ahmad R, Alabdallal A H, Aljafary M A, Al-Suhaimi E. (2018). Synthesis, characterization and antibacterial activity of silver nanoparticles using *Rhazya stricta*. *PeerJ*, 6: e6086. PubMed: 30588401 ;
<https://doi.org/10.7717/peerj.6086>
 15. Iqbal S, Bhangar M, Akhtar M, Anwar F, Ahmed K R, Anwer T. (2006). Antioxidant properties of methanolic extracts from leaves of *Rhazya stricta*. *Journal of medicinal food*, 9(2): 270-275.
<https://doi.org/10.1089/jmf.2006.9.270>
 16. Al-Dabbagh B, Elhaty I A, Al Sakkaf R, El-Awady R, Ashraf S S, Amin A. (2018). Antioxidant and anticancer activities of *Trigonella foenum-graecum*, *Cassia acutifolia* and *Rhazya stricta*. *BMC Complementary and Alternative Medicine*, 18(1): 1-12.
<https://doi.org/10.1186/s12906-018-2285-7>
 17. Marwat S K, Usman K, Shah S S, Anwar N, Ullah I. (2012). A review of phytochemistry, bioactivities and ethno medicinal uses of *Rhazya stricta* Decsne (Apocynaceae). *African Journal of Microbiology Research*, 6(8): 1629-1641.
<https://doi.org/10.5897/AJMRX11.024>
 18. Abadi F, Abdulaziz A, Hadhoud A, Baeshin N, Qari S, Alhejin A. (2011). An epidemiological survey and evaluation of the antimicrobial growth effect of *Rhazya stricta* (Decne) leaves extract on different genotypes of *Neisseria meningitides*. *Egypt J Med Microbiol*, 20(2): 77-86.
 19. Fazeli-nasab B, Moshtaghi N, Forouzandeh M. (2019). Effect of Solvent Extraction on Phenol, Flavonoids and Antioxidant Activity of some Iranian Native Herbs. *Scientific Journal of Ilam University of Medical Sciences*, 27(3): 14-26
<https://doi.org/10.29252/sjimu.27.3.14>
 20. Bautista-Trujillo G, Solorio-Rivera J, Renteria-Solorzano I, Carranza-German S, Bustos-Martinez J, Arteaga-Garibay R, Baizabal-Aguirre V, Cajero-Juarez M, Bravo-Patino A, Valdez-Alarcon J. (2013). Performance of culture media for the isolation and identification of *Staphylococcus aureus* from bovine mastitis. *Journal of medical microbiology*, 62(3): 369-376.
<https://doi.org/10.1099/jmm.0.046284-0>
 21. Talib W H, Mahasneh A M. (2010). Antimicrobial, cytotoxicity and phytochemical screening of Jordanian plants used in traditional medicine. *Molecules*, 15(3): 1811-1824.
<https://doi.org/10.3390/molecules15031811>
 22. Owuama C I. (2017). Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method. *African Journal of Microbiology Research*, 11(23): 977-980.
<https://doi.org/10.5897/AJMR2017.8545>
 23. Lambert R, Pearson J. (2000). Susceptibility testing: accurate and reproducible minimum inhibitory

- concentration (MIC) and non-inhibitory concentration (NIC) values. *J Appl Microbiol*, 88(5): 784-790. <https://doi.org/10.1046/j.1365-2672.2000.01017.x>
24. Pervin M, Hasnat M A, Lim B O. (2013). Antibacterial and antioxidant activities of *Vaccinium corymbosum* L. leaf extract. *Asian Pacific Journal of Tropical Disease*, 3(6): 444-453. [https://doi.org/10.1016/S2222-1808\(13\)60099-7](https://doi.org/10.1016/S2222-1808(13)60099-7)
25. Česonienė L, Jasutienė I, Šarkinas A. (2009). Phenolics and anthocyanins in berries of European cranberry and their antimicrobial activity. *Medicina*, 45(12): 992-999. PMID: 20173403
26. Shen X, Sun X, Xie Q, Liu H, Zhao Y, Pan Y, Hwang C-A, Wu V C. (2014). Antimicrobial effect of blueberry (*Vaccinium corymbosum* L.) extracts against the growth of *Listeria monocytogenes* and *Salmonella Enteritidis*. *Food control*, 35(1): 159-165. <https://doi.org/10.1016/j.foodcont.2013.06.040>
27. Rosaline X D, Sakthivelkumar S, Rajendran K, Janarthanan S. (2012). Screening of selected marine algae from the coastal Tamil Nadu, South India for antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine*, 2(1): S140-S146. [https://doi.org/10.1016/S2221-1691\(12\)60145-2](https://doi.org/10.1016/S2221-1691(12)60145-2)
28. Varier K M, Milton M, Arulvasu C, Gajendran B. (2013). Evaluation of antibacterial properties of selected red seaweeds from Rameshwaram, Tamil Nadu, India. *Journal of Academia and Industrial Research*, 1(11): 667-670.
29. Fazeli-Nasab B, Mirzaei N. (2018). Evaluation of total phenol and flavonoid content in a wide range of local and imported plants. *Scientific Journal of Ilam University of Medical Sciences*, 26(2): 141-154. <http://doi.org/10.29252/sjimu.26.2.141>
30. Kandhasamy M, Arunachalam K. (2008). Evaluation of *in vitro* antibacterial property of seaweeds of southeast coast of India. *African Journal of Biotechnology*, 7(12). <https://doi.org/10.5897/AJB08.120>
31. Taskin E, Caki Z, Ozturk M. (2010). Assessment of *in vitro* antitumoral and antimicrobial activities of marine algae harvested from the eastern Mediterranean sea. *African Journal of Biotechnology*, 9(27): 4272-4277.
32. Ghaffari M, Taheri A, Zobeidinezhad M. (2016). In vitro Evaluation of Antibacterial Effect of Ethyl Acetate Extract of Red Algae (*Gelidiella acerosa*) on Some Gram-positive and Gram-negative Bacteria. *Journal of Rafsanjan University of Medical Sciences*, 15(3): 209-222.
33. Rhimou B, Hassane R, José M, Nathalie B. (2010). The antibacterial potential of the seaweeds (*Rhodophyceae*) of the Strait of Gibraltar and the Mediterranean Coast of Morocco. *African Journal of Biotechnology*, 9(38): 6365-6372.
34. Kylli P, Nohynek L, Puupponen-Pimia R, Westerlund-Wikstrom B, Leppanen T, Welling J, Moilanen E, Heinonen M. (2011). Lingonberry (*Vaccinium vitis-idaea*) and European cranberry (*Vaccinium microcarpon*) proanthocyanidins: isolation, identification, and bioactivities. *Journal of agricultural and food chemistry*, 59(7): 3373-3384. <https://doi.org/10.1021/jf104621e>
35. Lacombe A, Wu V C, White J, Tadepalli S, Andre E E. (2012). The antimicrobial properties of the lowbush blueberry (*Vaccinium angustifolium*) fractional components against foodborne pathogens and the conservation of probiotic *Lactobacillus rhamnosus*. *Food microbiology*, 30(1): 124-131.

- <https://doi.org/10.1016/j.fm.2011.10.006>
36. Omar H, Shiekh H, Gumgumjee N, El-Kazan M, El-Gendy A. (2012). Antibacterial activity of extracts of marine algae from the Red Sea of Jeddah, Saudi Arabia. *African Journal of Biotechnology*, 11(71): 13576-13585.
<https://doi.org/10.5897/AJB12.780>
37. Lavanya R, Veerappan N. (2011). Antibacterial potential of six seaweeds collected from Gulf of Mannar of southeast coast of India. *Advances in Biological Research*, 5(1): 38-44.
38. Malayeri F A, Yazdanpour Z, Bandani H, Fazeli-Nasab B, Saeidi S. (2020). Antimicrobial and anti-biofilm effects of Thyme essential oils and Peppermint on *Acinetobacter baumannii* and *Staphylococcus aureus* resistant to different antibiotics. *New Findings in Veterinary Microbiology*, 2(2): 41-51.
<https://doi.org/10.35066/j040.2019.697>
39. Fazeli-Nasab B, Fahmide L. (2020). Evaluation of Antioxidant Properties and Phenolic Compounds of Different Mango (*Mangifera indica* L.) Southern Iran. *Crop Science Research in Arid Regions*, 2(1): 11-21.
<https://doi.org/10.22034/csrar.2020.119076>
40. Manion C R, Widder R M. (2017). Essentials of essential oils. *American Journal of Health-System Pharmacy*, 74(9): e153-e162.
<https://doi.org/10.2146/ajhp151043>
41. Aali E, Mahmoudi R, Kazeminia M, Hazrati R, Azarpey F. (2017). Essential oils as natural medicinal substances: review article. [Review Article]. *Tehran University Medical Journal*, 75(7): 480-489.
42. Nogueira J W A, Costa R A, da Cunha M T, Cavalcante T T A. (2017). Antibiofilm activity of natural substances derived from plants. *African Journal of Microbiology Research*, 11(26): 1051-1060.
<https://doi.org/10.5897/AJMR2016.8180>
43. Ribeiro-Santos R, Andrade M, Sanches-Silva A, de Melo N R. (2018). Essential oils for food application: natural substances with established biological activities. *Food and Bioprocess Technology*, 11(1): 43-71.
<https://doi.org/10.1007/s11947-017-1948-6>
44. Noorhosseini S A, Fallahi E, Samizadeh M, Beheshtipoor N. (2017). The Relative Priority of Medicinal Plants, Herbal and Chemical Medicines by Consumers Based on Economic and Treatment Criteria: Case Study of Rasht District. *Agricultural Economics Research*, 9(33): 71-99.

How to cite this article: Maryam Beigomi, Laleh shahraki-Mojahed, Batool Heydari-Sadegh, Narjes Dahmardeh, Reza Rouhani, Fereshteh Javadian*. Evaluation of Antimicrobial Activity of *Rhazya Stricta* (Apocynaceae) Extract Prepared with Different Solvents on *Staphylococcus Aureus* (Staphylococcaceae) Isolated from Humans. *International Journal of Advanced Biological and Biomedical Research*, 2021, 9(3), 241-253. Link: <http://www.ijabbr.com/article/244283.html>