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Evaluation of antibacterial activity of extract plant against *Staphylococcus aureus* and *Candida albicans* isolated from women

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ABSTRACT

Plants as sources of medicinal compounds from ancient times continue to play a major role in maintaining human health. The present study was performed to evaluate of antibacterial activity of *Rhazya stricta* extract prepared with different solvents on *Staphylococcus aureus* and *Candida albicans* isolated from women**.** The extracts of *Rhazya stricta* were prepared using a rotary device. The inhibitory concentration against *S. aureus* and *c.albicans* was determined using microdulition method. The results of this study showed that, the minimum inhibitory zone diameter of ethyl acetate extract against *S.aureus* was 1 mm and the maximum inhibitory zone diameter was 8 mm, the lowest inhibitory zone diameter of aqueous extract against *S.aureus* was 2 mm and the maximum inhibitory zone diameter of aqueous extract was equal to 12 mm. The results of this study showed that the highest inhibitory zone diameter was related to *R.stricta* extract prepared with ethanol solvent against *C.albicans*(18 mm) while the lowest inhibitory zone diameter was related to *R. stricta* extract prepared with ethyl acetate solvent (1 mm).The results of this study showed that the ethanolic extract of *R. stricta* showed the highest inhibitory properties of *S. aureus* and *C. albicans* that can be used in the treatment of infections caused by *S.aureus.*

1. Introduction

Staphylococcus aureus is a gram-positive and glucose-positive cocci of the *Staphylococcus* family and an opportunistic infection agent in humans[1]. This bacterium is of great importance due to the increase in antibiotic resistance and in addition is one of the most important and abundant causes of nosocomial infections worldwide[2]. *Staphylococcus aureus* is present as a human pathogen in 30% of the population and is one of the important causes of severe and deadly diseases. The clinical manifestations of *Staphylococcus aureus* are highly variable. Infections associated with this bacterium include: bacteremia, sepsis, pneumonia, osteomyelitis, and skin infections in humans [3].

Candida species most common fungal infections in humans and animal fungal infections in individuals who possess Factors such as cancer and leukemia, diabetes mellitus, prolonged therapy with antibiotics , AIDS and pregnancy, burns and transplantation They are, it is more common. The range of these infections varies from mucosal colonization to invasive and lethal infections. Among the various clinical forms of candidal infections, cutaneous and mixed candidiasis are more prevalent[4, 5].

Drug resistance, toxicity of these drugs, their solubility, stability and absorption are the most important problems. On the other hand, local infections are treated with nystatin, which has a very unpleasant taste.

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Therefore, the use of compounds with minimal side effects and having a desirable and acceptable taste in the treatment of lesions caused by Candida seems reasonable[6]. Other antifungal drugs used to treat various clinical forms of the disease include fluconazole azole, which has numerous side effects, including nausea, abdominal pain, vomiting, headache, and skin rashes[7-10].

Antifungal treatments using common antifungal drugs have several side effects and cause drug resistance and recurrent candidiasis infections[11, 12]. Therefore, the use of appropriate solutions to replace synthetic drugs that eliminate these problems has always been considered. One of these solutions is to use herbal medicines[13-15].

Rhazya stricta is a plant of the Apoynaceae family [16] and a subfamily of the Rauwolfioideae [17]. The drug has been used to treat diseases in Afghanistan, India, Iran, Iraq, Pakistan, Qatar and Saudi Arabia[18].

Raw ethanolic extract of *Rhazya stricta* fruit has antimicrobial activity [19]. Aqueous extract of *Rhazya stricta* showed antimicrobial activity against *Neisseria meningitides* [20]. *Rhazya stricta* and its metabolites are used to treat diseases such as cancer, skin diseases, hypertension, rheumatism, sore throat, syphilis and fever [21]. Various studies have shown that different parts of *R. stricta* contain many phytochemical elements such as alkaloids, flavonoids and terpenes[22].

The aim of this study was to investigate the antifungal activity of *R. stricta* extract prepared with different solvents on *Candida albicans* and *Staphylococcus aureus.*

2. Material and Methods

2.1 Plant Preparation:

After collecting the plants, they are rinsed with water and chopped for microbial tests. Afterwards they are dried for preparation of the plant extract in the shadow.

2.2 Extract Preparation

Rhazya stricta collected from the mountainous regions of Iran (Saravan) and then chopped. For the extract preparation, 10 g of dry powder of the plant was placed in a half-liter of erlens containing 100 mL of Water, Ethanol, Methanol, Hydroalcoholic. The content of erlens was mixed at room temperature for 24 hours by a shaker device with 130 rpm speed and was then filtered using Whatman paper No. 2. The solvent was then separated from the extract by a rotary device and by a vacuum pump (vacuum distillation). The obtained extract was weighted and then dissolved in DMSO solvent and it was maintained in the refrigerator at 4°C for use.

2.3 Isolation of *Staphylococcus aureus*

10samples from 100 persons were collected from gynecological urinary tract infection in hospital Zabol- iran. The samples were quarterly collected from infected men. Ten microliters of each sample were cultured on blood agar. Isolated Gram and catalase positive cocci were further tested for biochemical characterization.

2.4 Isolates of *Candida albicans*:

After sampling the vaginal using the sterile swap and Falcon tube by the gynecological specialists, 4samples were isolated and transferred to the laboratory and cultivated on agar dextrose saburo and broth dextrose saburo according to the manufacturer's instructions. After the growth of each sample, lam was prepared and the candidate samples were identified. Colonies of *Candida albicans* were prepared in the media of agar dextrose saburo at 37°C in homogenous suspension sterile physiology serum and the rate of the light passing of the suspension was measured using the spectrophotometry device with 530 nm. The rate of the passing light of 90% is necessary for preparing a suspension with nearly 10^6fungi cells per mL. For determining the inhibitory concentration of the extracts, incubation in media was used (the concentration of 25, 50 and 100 ppm were used). Finally, they were placed in the incubator and the samples were analyzed after 24 - 48 hours.

2.5 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of plant extracts:

The broth microdilution method was used to determine MIC and MBC .All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/ v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 0.3 mg/ml to 10.00 mg/ml. To each well, 10 µl of indicator solution (prepared by dissolving a 10-mg extract in 2 ml of DMSO) and 10 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension (10^6 CFU/ml) was added to each well to achieve a concentration of 10^4 CFU/ml. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plated were prepared in triplicates, and then they were placed in an incubator at 37°C for 18–24 hours. The colour change was then assessed visually. The lowest concentration at which the colour change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC and MBC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the extract at which the incubated microorganism was completely killed.

3. Result

The results of this study showed that the minimum inhibitory zone diameter of ethyl acetate extract of *Rhazya stricta* on *Staphylococcus aureus* was 1 mm and the maximum inhibitory zone diameter was 8 mm, the lowest inhibitory zone diameter of aqueous extract was 2 mm and the maximum inhibitory zone diameter of aqueous extract was equal to 12 mm. The maximum inhibitory zone diameter of ethanolic extract was 18 mm and the minimum inhibitory zone diameter was 7 mm, the maximum inhibitory zone diameter of methanolic extract was 12 mm and the lowest inhibitory zone diameter was 4 mm while the maximum The diameter of the inhibitory zone of the hydraulic extract was equal to 15 mm and the minimum diameter of the inhibitory zone was 5 mm(Figure 1; Figure 2).

The lowest inhibitory concentration of ethyl acetate extract of *Rhazya stricta* on *Staphylococcus aureus* was equal to 12.5 ppm in which two strains were inhibited and the highest inhibitory concentration of ethyl acetate extract was 100 ppm in which 3 strains were inhibited. The lowest inhibitory concentration of aqueous extract was equal to 6.25 ppm in which one strain was inhibited and the highest inhibitory concentration was 50 ppm in which 2 strains were inhibited in this concentration. The lowest and highest inhibitory concentrations of ethanolic extract were 6.25 and 50 ppm, which were inhibited by 2 and 1 strains, respectively. The lowest and highest inhibitory concentrations of methanolic extract were 6.25 and 50 ppm, in which 1 and 3 strains were inhibited. The lowest and highest inhibitory concentrations were 6.25 and 25 ppm, in which 3 and 5 strains were inhibited (Figure 1; Figure 2).

The results of this study showed that the highest inhibitory zone diameter against *C.albicans* was related to *R.stricta* extract prepared with ethanol solvent (18 mm) while the lowest inhibitory zone diameter was related to *R. stricta* extract prepared with ethyl acetate solvent (1 mm) (Figure 3).

The lowest inhibitory concentration of ethyl acetate extract of *Rhazya stricta* extract on *Staphylococcus aureus* was 50 ppm in which two strains were inhibited and the highest inhibitory concentration was 100 ppm in which two strains were inhibited in this concentration. The lowest inhibitory concentration of aqueous extract was 12.5 ppm in which 1 strain was inhibited and the highest inhibitory concentration was 50 ppm in which 1 strain was inhibited in this concentration (Table 1).

The lowest concentration of ethanolic extract against *C.albicans* was 6.25 ppm in which 1 strain was inhibited, while the highest inhibitory concentration was 25 in which 1 strain was inhibited. The lowest inhibitory concentration of methanol extract against *C.albicans* was 12.5 ppm in which 1 strain was inhibited and the highest inhibitory concentration was 50 ppm in which 1 strain was inhibitory concentration. The lowest inhibitory concentration of hydroalcoholic extract was equal to 6.25 in which 1 strain

was inhibited and the highest inhibitory concentration was equal to 25 ppm (Table 2).

Table 1. Minimum inhibitory concentration and minimum Bactericidal concentration of *Rhazya stricta* extract on *Staphylococcus aureus* (ppm)

Strain bacterial	Hydroalcoholic	Methanol	Ethanol	Water	Ethyl acetate
1	12.5-25	12.5-25	6.25-12.5	25-50	50-100
2	12.5-25	25-50	12.5-25	25-50	100-200
3	6.25-12.5	25-50	12.5-25	12.5-25	50-100
4	25-50	50-100	25-50	50-100	100-200
5	25-50	12.5-25	12.5-25	12.5-25	12.5-25
6	6.25-12.5	50-100	25-50	6.25-12.5	25-50
7	25-50	6.25-12.5	50-100	12.5-25	12.5-25
8	25-50	12.5-25	12.5-25	50-100	50-100
9	25-50	25-50	6.25-12.5	12.5-25	100-200
10	6.25-12.5	50-100	12.5-25	25-50	50-100

Table 2. Inhibitory zone diameter of *Rhazya stricta* extract prepared with different solvents on *Candida albicans* (mm)

Strain	Hydroalcoholic	Methanol	Ethanol	Water	Ethyl acetate
C.albicans1	12.5-25	12.5-25	6.25-12.5	25-50	50-100
C.albicans 2	12.5-25	25-50	12.5-25	25-50	100-200
C.albicans 3	6.25-12.5	25-50	12.5-25	12.5-25	50-100
C.albicans 4	25-50	50-100	25-50	50-100	100-200

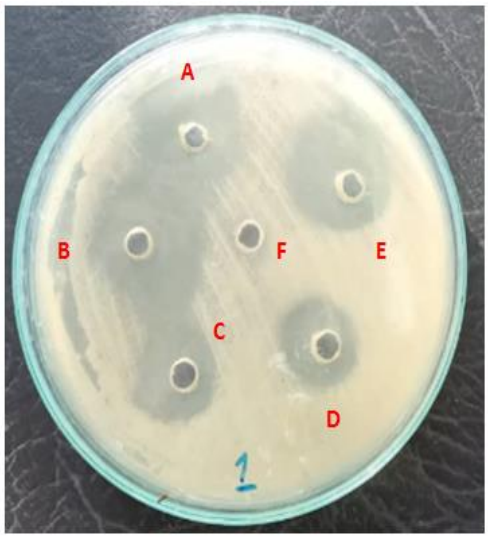

Fig. 1. Inhibitory zone diameter of *Rhazya stricta* extract prepared with different solvents (Ethyl acetate(D), Water(C), Ethanol(E), Methanol(B), and Hydroalcoholic(A), and Control (F)) on strain 1 of *Staphylococcus aureus* (mm)

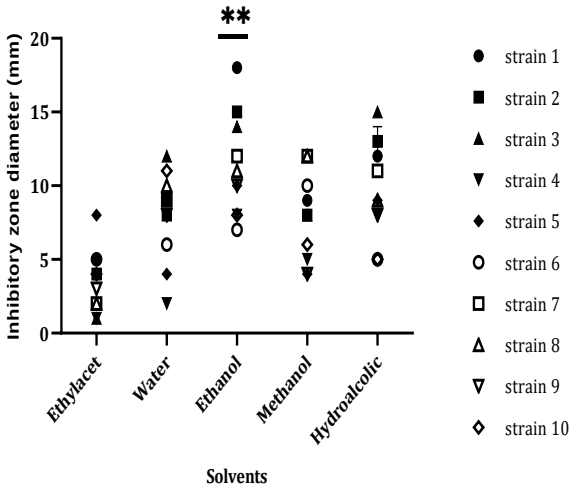

Fig. 2. Inhibitory zone diameter of *Rhazya stricta* extract prepared with different solvents on all strains of *Staphylococcus aureus* (mm)

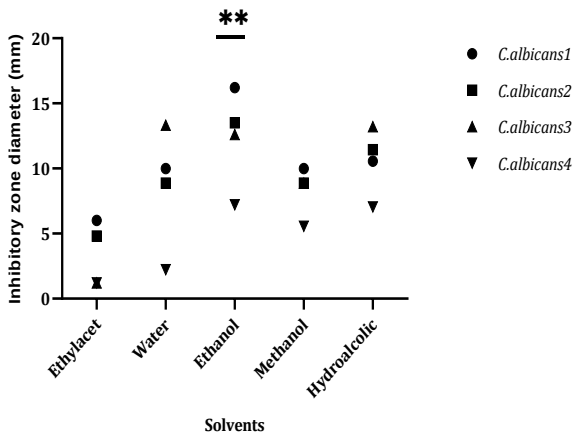

Fig. 3. Inhibitory zone diameter of *Rhazya stricta* extract prepared with different solvents on all strains of *Candida albicans* (mm)

4. Discussion

Iran, with its five major climates, has been a unique land for growing more than 7,500 plant species, many of them are categorized as herbal medicine [23]. Herbal medicines, as a basement of treatment of various diseases and ailments, have been described by ancient well known Persian medical scholars including Rhazes, Heravi, Avicenna and many others (19).

In a study by Khan et al., Which examined the antimicrobial activity of the aqueous extract of the alkaloid *Rhazya stricta*, the results showed that the alkaloid extract had a good effect against *Escherichia coli* and methicillin-resistant *Staphylococcus aureus*[24].

In the study of Shehzad et al., The synthesis of nanoparticles in the extract of *Rhazya stricta* root, performing antimicrobial activity, showed that the synthesized nanoparticles increased the antimicrobial activity against *Escherichia coli* compared to *Bacillus subtilis*[25].

In the study of Khan et al., the antifungal activity of *Rhazya stricta* methanolic extract showed that the diameter of the inhibitory zone against *T. Longifusis, C. albicans, F. solani* was equal to 25 ± 0.5, 23 1 1 and 1.5 ± It was 18 mm while the diameter of the inhibitory aura of chloroform extract against *T. longifusis, A.flavus* and *M. canis* was 10 ‹0.5, 7 ± 1 and 11 ± 1.5 mm [26].

In a study by Kabi et al., Which examined the antimicrobial activity of *Rhazya stricta* aqueous extract on broad-spectrum beta-lactamase-producing bacteria, the results showed that the highest inhibition rate of beta-lactamase-producing bacteria (49.2%) compared to non-beta-lactamase-producing bacteria (50.8%)[27].

The study of Jahani, The results show that highest MIC of *R. officinalis* against *C. albicans* was 100 ppm and low MIC was 12.5 ppm against *C. albicans* and the highest MIC of *E. platyloba* against *C. albicans* was 150 ppm and low MIC was 12.5 ppm against *C. albicans*[28].

R. stricta chloroformic and methanolic root fractions demonstrated antifungal activities against *Aspergillus terreus, Aspergillus flavus*, and *Candida albicans* [29]. Another study revealed that fractionated *R. stricta* methanol and chloroform samples showed antifungal activity against *Trichophyton longifusis, C. albicans, A. flavus*, and *Fusarium solani* [26].

In vitro, *R. stricta* leaf and fruit extract demonstrated antibacterial activity against *Staphlococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Streptococcus pyogenes*, and *Salmonella typhi* [19]. *R. stricta* leaf extract demonstrated a control of bacterial growth on locally isolated meningococcal strains that increased with concentration and treatment time [20]. Chloroformic and methanolic extracts of *R. stricta* roots exhibited antimicrobial activity toward *B. subtilis, E. coli, S. aureus,* and *P. aeruginosa.* Tetrahydrosecamine was purified from the plant and demonstrated a wide range of antibacterial activity (effective toward all bacteria except *E. coli*; MIC values ranged from 0.1 to 5.0 mg/mL). Another active substance, strictanol, was also shown to be effective against P. *aeruginosa* and *E. coli* (MIC 0.5 mg/mL for both microbes)[29]. The Ag nanoparticles synthesized using silver nitrate and methanol root extract of *R. stricta* showed improved antibacterial activity against *B. subtilis* and *E. coli* [25]. At low concentrations, the tested R. stricta extract mixed with Ag nanoparticles inhibited the growth of several pathogenic bacteria, including *Klebsiella pneumoniae, B. subtilis,* and *S. typhi* [30].

5. Conclusion

The results of this study showed that *Rhazya stricta* extract with all its solvents was an inhibitor of *Candida albicans* and the best extract was ethanolic extract of *Rhazya stricta*. The phytochemistry profile of *R. stricta* contains a unique alkaloid content that has been isolated and identified significantly, and we found that the non-alkaloid contents need more investigation. R. stricta extract has shown pharmacological activity such as antimicrobial, anticancer, antidiabetic, and antioxidant activities, as well as biological activity such as insecticide, allelopathic, and soil remediation activities. Some pharmacological aspects, such as the antiviral activity of the plant extract have not been examined yet.

Abbreviation

Not applicable

Conflict of interest

The authors declare there is no conflict of interest in this research study.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All data generated during this study are included in this published article.

Ethics approval and consent to participate

No human or animals were used in the present research.

Ethics declarations

The authors declare no conflict of interest in financial or any other sphere.

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