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Research Article

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Antimicrobial activity of *Rumex nervosus* extract collected from Yemen against local selected isolates pathogens

Abstract

Rumex nervosus is a genus of plant belongs to the family *polygonaceae*. In this study *rumex nervosus* represent as one of the selected plants used as a treatment for many diseases such as inflammatory and painful in Yemen. Extraction of this plant was by ethanolic and methanolic solvents. The obtained extract was tested for its antimicrobial activity against two Gram-positive, three Gram-negative and *Candida albicans* using agar diffusion method. However, *S. aerus*, *E-Coli* and *Candida albicans* showed the antimicrobial activity for ethanolic and methanolic solvents in (150mg).The results showed that this plant has antimicrobial activity against bacteria and fungi, so this plant is potential to be used as antimicrobial.

Keywords: *Rumex nervosus*, antimicrobial, ethanol, methanol, DMSO, Yemen

Volume 8 Issue 3 - 2020

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Received: April 27, 2020 | **Published:** May 13, 2020

Introduction

Rumex nervosus is a genus of plant belongs to the family of Polygonaceae that used traditionally worldwide to treat different diseases related to microbial infection. It has ingredients that can be used to treat chronic as well as infectious diseases.[1] Many infectious diseases treated with herbal remedies throughout the history of humankind. In recent years, the reports indicated that more than 80% of the world's population relied on traditional medicine for their primary healthcare needs also the use of natural antioxidants present in plant and other biological materials has attracted considerable interest due to their presumed safely, nutritional and therapeutic value.[2,3]

Medicinal plants considered the sources of bioactive compounds used mainly for medicinal purposes. Recently, human pathogenic microorganisms have developed resistance in response to the indiscriminate use of commercial used antimicrobial drugs commonly employed in the treatment of infectious diseases.[4] Herbal medicines are a source of primary health care owing to their attributes having wide biological and medicinal activities in developed and developing countries. This herbal medicine might be safe and would overcome resistance produced by the pathogens, as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell.[5,6]

Testing of medicinal plants for antimicrobial activity is important for finding potential new compounds for therapeutic use.[7] *Rumex nervosus* is a perennial herb mainly distributed in Saudi Arabia, Ethiopia, Somalia, Kenya, Tanzania, Eritrea and Yemen.[8,9] Yemen is one of the developing countries depend on plant resources mainly for herbal medicines. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries.[10,11] Actually, *Rumex nervosus* used in folk medicine by Yemeni people was selected to determine its antimicrobial activity. Therefore, the aim of the present study was to evaluate the antimicrobial activity of extract from medicinal plant *R. nervosus* used in folk medicine against five pathogenic bacteria and fungi.

Materials and methods

Plant collections

The *Rumex nervosus* was collected from Sana'a city, Yemen, in February 2020. Sample was air dried at room temperature, powdered and stored in dark colored bottles and protected from light prior to further use. The analysis has been carried out in March 2020 at the Laboratory of science Faculty of science Sana`a University Yemen.

Extraction technique

Sample was finely ground using an electrical grinder at speed 6 for 2 min and ground sample (5g) of sample was soaked for five days in ethanol and methanol at 1:5 ratio at room temperature. The mixture of samples and solvent was filtered through a filter paper (Whattman No. 2). The solvent was removed by drying at the oven at 40°C. The extract was transferred into glass sealed amber dark bottles and then stored in at 4°C for subsequent analyses.

Antibacterial activity

Five bacterial and one Fungi were used for the study. Gram positive bacteria include of Bacillus subtilis Staphylococcus aerus and Gram negative bacteria include *Escherichia coli*, *Pesudomonas aeruginosa* and *Kelibsella sp*. All the tested strains were local isolates and were obtained from Department of Biology, Division of Microbiology, Faculty of Science, Sana'a University. These bacteria served as test pathogens for antibacterial activity assay. Three different concentrations of each extract of selected plants (50, 100 and 150mg/ml) were dissolved in 10% dimethyl sulfoxide (DMSO) in purified water to be used in antimicrobial activity test. Extract solutions were prepared just before carrying out the test. Antibacterial activity of the extracts was determined by agar well diffusion method.

The bacterial suspensions containing 106 CFU/ml of bacteria were spread on petri dishes plates with a sterile swab moistened with the bacterial suspension.

In each of these plates, five wells were cut out using a standard corn borer (7mm). About 60µl of each extract was added into different wells (duplicate each concentration), DMSO was used as a negative control. Positive control antibiotic wells were placed in the plate. All the plates were incubated for 24h at 37°C. After incubation bioactivity was evaluated by measuring the zone of inhibition. The experiment was performed in two of antibiotics standard Gentamycin and Nystatin (10 mcg) were used as reference to determine the sensitivity of each bacterial and fungal species tested and used as control positive.

Results

To our knowledge the extract of *R. nervosus* were tested with different concentrations value (150mg, 100mg and 50mg) for antimicrobial effect against Gram-positive such as (*S. aureus*, and *B. subtilus*), Gram-negative likewise (*E.coli, k. pneumonia* and *P. aeruginosa*) bacteria, as well as antifungal activity against *C. albicans* strain. After 24 h of incubation, the antimicrobial activity of the extract of sample and the dilutions were plated on agar plates to determine the presence or absence of inhibition zone and by measuring the diameter of the inhibition zone around the wells.

When aqueous extract was tested by agar diffusion method, the results showed that among Gram-positive microorganisms, *R. nervosus* has dose-dependent growth inhibitory activity of *S. aureus* in methanolic solvent, while *B. subtilus* was found inhibitory activity in ethanolic solvent. With Gram-negative strains, only *E. coli* had a highly growth inhibitory in all solvents, while other gram negative used such as *k. pneumoniae* and *P. aeruginosa* were in a close of growth inhibitory in both ethanolic and methanolic solvents. Furthermore, *C. albicans* revealed the highest growth inhibitory for all solvents.

Generally, the antibacterial activity of *R. nervosus* extract appears to be more inhibitory to Gram-positive bacteria than Gram-negative bacteria. The zones of growth inhibition on the Gram-positive, Gram negative and *C. albicans*, that used in this research showed the growth inhibition in Table 1. The concentration of *R. nervosus* extracts was determined by incubating three concentrations (150 mg, 100mg and 50mg) of extracts with a standard inoculum of microbial cultures; however, 150 mg was the highest concentration showed the effects, while placebo showed no growth inhibitory on all microorganisms. Gentamycin and was the only antibiotics used as standard for gram positive and gram-negative bacteria. While, Nystatin was used as standard for *Candida albicans.* These standards revealed antimicrobial activity with all organisms in ethanolic and methanolic solvents (Figure 1 & 2).

Table 1 Showed that the zones of *Rumex Nerovsus* by using ethanol and methanol solvents

Conc & zone	Ethanol					Methanol				
	Zones/mm			Standard	Plac*	Zones/mm			Standard	Plac*
Organisms	150 mg	100 mg	50 mg	Gentamycin		150 mg	100 mg	50 mg	Gentamycin	
Staphylococcus aerus	15 mm	14 mm	12 mm	18 mm	NG*	16 mm	15 mm	14 mm	20 mm	NG*
Bacillus subtilus	16 mm	15 mm	14 mm	17 mm	NG*	15 mm	14 mm	13 mm	17 mm	NG*
Pseudomonas aeruginosa	16 mm	12 mm	12 mm	16 mm	NG*	17 mm	15 mm	14 mm	17 mm	NG*
E- Coli	20 mm	16 mm	15 mm	20 mm	NG*	20 mm	17 mm	16 mm	21 mm	NG*
klebsiella pneumoniae	15 mm	12 mm	11 mm	17 mm	NG*	14 mm	13 mm	12 mm	16 mm	NG*
Candida albicans	21 mm	19 mm	19 mm	18 mm Nys*	NG*	21 mm	20 mm	19 mm	20 mm Nys*	NG*

*NG, no growth; Plac, Placebo; Nys, Nystatin

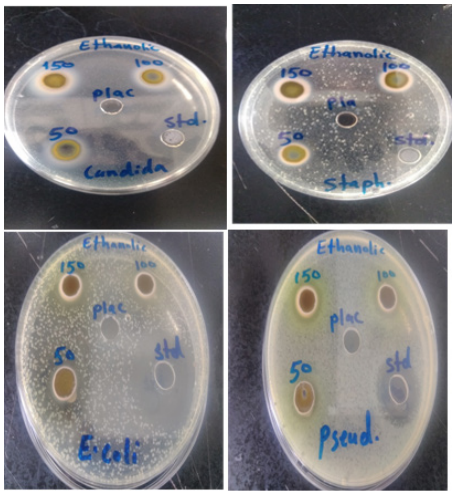

Figure 1 Inhibition zones observed with leaf ethanolic extracts of Rumex nervosus.

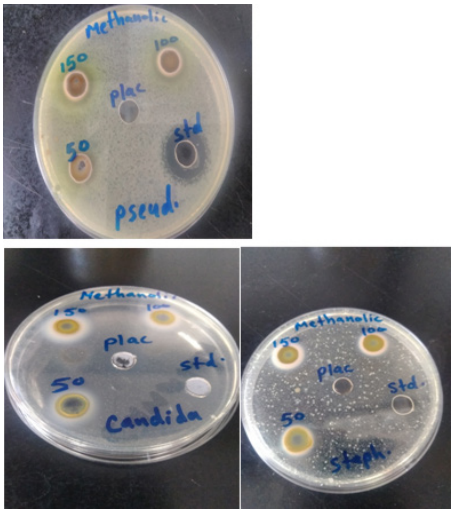

Figure 2 Inhibition zones observed with the leaf of methanolic extract of Rumex nerovsus.

Citation: Al-Nowihi M, Faisal A, Al-Asbahi G. Antimicrobial activity of *Rumex nervosus* extract collected from Yemen against local selected isolates pathogens. *J Microbiol Exp.* 2020;8(3):93–96. DOI: 10.15406/jmen.2020.08.00291

Discussion

To the best of our knowledge, the search for herbal recourse owning antimicrobial characteristics have been increased due to their possibility uses in several chronic and infection disease. Our study has been focused in particular on the famous medicinal plant used in Yemen. This current study used methanolic and ethanolic solvents to extract this plant against gram positive and gram-negative bacterium and evaluated obtained extract for antimicrobial activity using well diffusion assay.

This study showed that the highest antimicrobial activity was with methanolic solvent with all organisms. The *Rumex nervosus* indigenous to Yemen, locally known as Othorub, is traditionally used as an excellent dressing for wounds, orthopedic fractures, inflammatory and painful condition in Yemen. The study conducted by Al- Dubaie et al.,[12] reported that this species reveal antimalarial properties. In addition, the results of another study conducted in Ibb city indicated that the *Rumex nervosus* growing in Yemen can be used in the future as a source of active antimicrobial agents for industry and medicine.[13] It is the first report about antimicrobial activity of bacterial and fungal pathogens includes *Staphylococcus aerus, Bacillus subtilus, Pseudomonas aeruginosa, E- Coli, klebsiella pneumonia and Candida albicans*.

Our present study showed that the methanolic fraction found a high antimicrobial effective against several gram positive and gram negative bacteria including *S. auerus, E- Coli, P. aeruginosa and C. albicans,* while other bacteria had the same antimicrobial activity. Our finding was agree with previous studies conducted in Ethiopia, reported to possess antibacterial activity against *S. aureus*[14] and similar with study by Al-Asmari et al.,[15] who reported that the methanol extract of Remux nervosus had shown antibacterial activity against *S. aureus*.[18] While disagree with the study by Al-Asmari et al.,[15] who revealed that no activity on the *C. albicans.* The results of our study of extract from *Rumex nervosus* have a good antibacterial activity against *E- Coli.* This finding was different with other studies revealed that the least activity was recorded against *E. coli.*[7,16] In addition, our results was different with a study conducted by Kasimala et al.,[17] on methanol extract of *Rumex nervosus* had also showed no antibacterial activity against *E.coli* and *S. aureus.* Where as, our results reported that highly antibacterial activity against *E.coli* and *S. aureus.* In this study, the extract of *Rumex nervosus* showed better antibacterial activity against some of the pathogens.

In this study, ethanolic fraction showed better antimicrobial activity against *Candida albicans, E- Coli, Bacillus subtilus, and Pseudomonas aeruginosa,* [21mg, 20mg, 16mg, 16mg] respectively, whereas other organisms likewise *K. pneumonia* and *S.auerus* were equal in their microbial activity in concentration of 150mg. No previous studies showed their results in ethanolic solvent due to it does not gave a better results compared with methanol. Moreover, our research showed a high antimicrobial activity with all organisms using gentamycin as standard. The zone of gentamycin was similarity with a highly concentration [150mg]. Furthermore, Nystatin was used as standard for antifungal (*Candida albicans*) and showed a high activity with all solvents. Generally, the antibacterial activity of *R. nervosus* extract appears to be inhibitorier in methanol than ethanol due to ethanol colorless, volatile liquid.

Conclusion

In conclusion, this recent study displayed that the extract of this medicinal plant has antimicrobial activity with *E. Coli* and *S. aureas* of Gentamycin and efficiency with *Candida albicans* for Nystatin by using two solvents. However, the methanolic solvent extract of *R. nerovsus* had more efficacy against Gram-negative and Gram-positive bacteria. From the present result, we recommend further investigation on the fractionated components using different solvent and standard medium.

Acknowledgments

The authors are grateful to Mr. Abdu Faisal who helped us for collecting the plant in Sana`a, Yemen, and providing facilities to do this work.

Conflicts of interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Citation: Al-Nowihi M, Faisal A, Al-Asbahi G. Antimicrobial activity of *Rumex nervosus* extract collected from Yemen against local selected isolates pathogens. *J Microbiol Exp.* 2020;8(3):93–96. DOI: 10.15406/jmen.2020.08.00291