

The Study of the Effect of Aqueous and Alcoholic Extracts of Indigenous Medicinal Plant of Arasbaran of Intestinal Bacteria

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ABSTRACT

The purpose of this research was to study the effects of aqueous and alcoholic extracts of *Nasturtium officinale L.* on the intestinal bacteria. Firstly we prepared the aqueous and alcoholic extracts of *Nasturtium* plant in soxhlet method. Then the dilutions of 20, 30, 50 and 100 milligram per milliliter of these extracts were prepared and used against the standard strains of *Escherichia coli* and *Enterococcus faecalis* in standard way and by disk diffusion method and MIC determination. The findings showed that the aqueous extract had a little effect close to zero on the bacteria. While, the alcoholic extract had more significant effect on both of the bacteria. *Escherichia coli* was more sensitive than *Enterococcus faecalis*. Intracellular tests seem necessary before the clinical usage of this extract.

KEY WORDS: *Nasturtium officinale L.*, *Escherichia coli*, Arasbaran, Disk Diffusion Agar, *Enterococcus faecalis*

INTRODUCTION

Enterococci are negative catalase Gram-positive cocci and they are able to grow in the presence of 6.5 percent of salt and 40 percent of bile salts. These organisms are considered as the normal flora of the intestine in warm-blooded animals such as human. The most common Enterococci involved in the human infections are *Enterococcus faecalis* (85 – 90) percent and faesium (5 – 10) percent which cause the urinary tract infections, wounds and endocarditis [1]. The Gram-negative *Escherichia coli* bacteria belongs to the Enterobacteriaceae family and has greatly grown and appeared in the form of new resistant strains as one of the main factors in creation of urinary tract, respiratory apparatus, digestive tract and nosocomial infections [2]. This bacterium is considered as the most dominant factor of urinary infections, the most common factor of travel diarrhea and one of the most important pathogens causing nosocomial infections. The presence of this bacterium in the normal flora of the intestine of the society's people and the nosocomial spaces has raised it as one of the main sources of transmission of drug resistance [3].

Nasturtium plant with the common scientific name of *Nasturtium officinale L.* is a plant belongs to Cruciferae or Brassicaceae family. The main home of this plant was the central and western Europe but today, it has been spread all over the world such as Asia, Europe and all over North America and it has a long history as a medicinal plant. *Nasturtium* has many chemical and influential compounds like quercetin flavonoids, beta-carotene carotenoids, lutein, xi-xanthine and vitamin C. Quercetin has anti-fungal and anti-bacterial properties [4]. The anti-lung-cancer effect has been proposed for this plant in new studies [5]. Also this plant inhibits the release of histamine and increases the intracellular free concentration in Calcium and decreases the oxidative metabolites of acetaminophen [6].

The current study tries to investigate the antibacterial property of the *Nasturtium officinale L.* against *Escherichia coli* and *Enterococcus faecalis* bacteria.

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MATERIALS AND METHODS

This experimental-laboratory research was done in 2014 in the microbiology laboratory of Islamic Azad University of Ahar. The medicinal plant *Nasturtium* with the scientific name of *Nasturtium officinale L.* was collected from meadows and forests of Arasbaran and was cooled far from sunlight and in room temperature. The aqueous and alcoholic extracts of the plant were extracted in soxhlet method. 5% dimethyl sulfoxide was prepared from the obtained extracts in concentrations of 20, 30, 50 and 100 mg/ml and it was used against the selected intestinal bacteria in disk diffusion method and MIC/MBC determination.

Distilled water was used in order to prepare the aqueous extract and pure methanol was used to prepare the alcoholic extract. The bacterial suspension of 0.5McFarland was developed through the Muller-Hinton Agar medium in disk diffusion method to do Antibiogram. After that blank disks were placed in the concentrations of the prepared extract for 1 hour for the extract to be absorbed by these disks. Then, disks were incubated for an hour in order to be dried and then they were placed 2 centimeters distant from each other on the medium. The plates were incubated for 24 hours and after that they were investigated in terms of bacteria growth inhibition zone [7].

Also the dilutions of 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 mg/ml were prepared in order to determine MIC. Then the bacterial suspension was added to the pipes and they were incubated for 24 – 48 hours. After this time, the pipe with the least amount of extract and no opacity was considered as MIC. In order to determine it, MBC was transferred from the pipes without growth to plates with Muller-Hinton Agar and was inoculated, the plates were investigated after 24 – 48 hours of incubation and the plate with the least concentration of the extract and no colony had grown in it was considered as MBC [8].

RESULTS

The results obtained from this research prove the antibacterial effect of methanol and aqueous extract of the *Nasturtium officinale L.* on both of the pathogenic intestinal bacteria of *Escherichia coli* and *Enterococcus faecalis*. The results are shown in tables 1 and 2.

Table 1. The Inhibition zone diameter of bacterial growth in disk diffusion and MIC/MBC amount of the aqueous extract of *Nasturtium officinale L.*

MBC	MIC	Positive control (Gentamicin)	Negative control (Distilled water)	100	50	30	20	Aqueous extract Bacteria
100	50	20	—	8	5	—	—	<i>Escherichia coli</i>
—	100	18	—	4	—	—	—	<i>Enterococcus faecalis</i>

*The extract concentration and MIC and MBC amount are in milligram per milliliter

*The inhibition zone diameter is in millimeters

Table 2. The Inhibition zone diameter of bacterial growth in disk diffusion and MIC/MBC amount of the Methanolic extract of *Nasturtium officinale L.*

MBC	MIC	Positive control (Gentamicin)	Negative control (Distilled water)	100	50	30	20	Aqueous extract Bacteria
12	6.25	20	—	19.32	16.64	14	13	<i>Escherichia coli</i>
50	25	18	—	17	14.20	12	10	<i>Enterococcus faecalis</i>

*the extract concentration and MIC and MBC amount are in milligram per milliliter

*The inhibition zone diameter is in millimeters

DISCUSSION

Herbal essence and extract is used in foods, drugs and natural therapies supplements for more than thousands of years [9]. The plant extracts are new sources of the antibacterial compounds against the pathogen bacteria and the library studies have determined the effect of herbal on stopping the bacterial growth so that this effect can be classified in three categories of weak, average and strong.

The results of the current study indicate that the aqueous extract of the *Nasturtium* plant has a little influence on the desired bacteria and they just indicated the growth inhibition zone in high concentrations. The effect of this extract on *Escherichia coli* bacteria is a little more compared to *Enterococcus faecalis* bacteria. Also after investigating the effect of methanol extract of the *Nasturtium* plant, it can be said that this extract has a significant influence on both of the bacteria so that in higher dilutions like 100 milligram per milliliter the zone has a similar inhibition growth with the gentamicin antibiotic.

Also it can be concluded from the results of this extract that *Escherichia coli* bacteria was more sensitive than *Enterococcus faecalis* bacteria. Different studies have been done so far about the anti-cancer, anti-diabetic and other properties of the Nasturtium plant and also a significant number of studies have been done about the influence of different extracts on the intestinal bacteria.

In 2007, Shahrokhi et al. indicated that using the aqueous extract of the Nasturtium plant decreases the glucose of plasma in the diabetic animal [10], while in this research the antibacterial effect of the aqueous extract of Nasturtium plant was examined.

In another study, Dadash Beygi et al. (2010) investigated the antibacterial effect of basil plant on *Escherichia coli* and *Pseudomonas aeruginosa* and the results of that study also proved the influence of the Nasturtium plant extract on *Escherichia coli* [7].

Conclusion

After doing this practical work, it can be concluded that the methanol extract of Nasturtium can be an influential opening in treating intestinal diseases. Intracellular tests (in vivo) seem necessary before the clinical usage of this extract.

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REFERENCES

1. Saeed, AK. Mohammad, SM. Ashraf, AK., Selective isolation of multi drug resistant *Enterococcus* spp. From poultry and dairy farms: detection of rifampin and vancomycin resistance gene markers by PCR, Molecular Cellular Probes, 2005, 19: p 27-34.
2. Nield, BS. Holmes, AJ. Gillings, MR. Stakes, HW., recovery of new integron classes from environmental DNA, FEMS Microbiology Letters, 2001, p 195:59-65.
3. Tangden, T, Cars, O. Mihus, A. Lpwidin, E., foreign travel is a major risk factor for colonization with *E. coli* producing CTX-M-type extended spectrum β - lactamases: a prospective study with Swedish Volunteers, Antimicrobial Agents and Chemotherapy, 2010, 54: p 3564-3568.
4. Onyimanyi, A.E. Olabade, A. Okeke, G.C., performance and economic characteristics of Broilers fed varying dietary levels of neem leaf meal, International Journal of Poultry Science, 2009, 8(3): p 256-259.
5. Goda, Y. Hoshine, K. Akiyama, Y. Ishikawa, T., constituents in water extract: inhibitors of histamine release from RBL-2H3 cells induced by antigen stimulation, Biol Pharm Bull, 1999, 22 (12): p 1319-1320.
6. Dehghani, G. Ahmadi, S. Ranjbar, G., effect of sulfate and vanadate on glucose homeostasis in rats with severe diabetic, Academic Journal of Rafsanjan Medical Sciences University, 2000, 3(1): p 170-176.
7. Dadash Beygi, M. Rezakhani, V., Pashdar, M., Darabi, A., Sorour, A., The Study of the Antibacterial Effect of Basil Plant Extract on *Escherichia coli* and *Pseudomonas aeruginosa*, Journal of Veterinary, Islamic Azad University, 2010, 4(4): p 71 – 80.
8. Alizadeh, H., Tayeri, T., Gholipour, S., Alizadeh, Sh., Izadpanah, M., Comparing the Antibacterial Effects of Methanol Extracts of Root and Leaves of Turnip Plant on Some of the Foodborne Bacteria, National Electronic Conference of Basic Sciences, Tehran, Earth Explore Research Center, 2014, p 35.
9. Joshi, B. Lokhak, S., Antibacterial property of different Medical plants, Katmandu University Journal of Science, Engineering and Technology, 2009, 5(1): p 143-150.
10. Shahrokhi, N., Hadad, M., Sha'bani, M., Heydari, M., Effect of Oral Administration of Nasturtium Plant in Controlling Sugar and Lipid in Diabetic Rats, Journal of Medical University of Rafsanjan, 2007, 6(4): p 245 – 254.