

EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF THE SEED HULL OF QUERCUS BRANTII ON SOME GRAM NEGATIVE BACTERIA

Azar D. Khosravi¹, Atoosa Behzadi²

ABSTRACT

Objective: Due to rapid increase of antibiotic resistance especially among gram negative bacteria that cause diarrheal diseases, the role of methanol extract of oak seed hull (*Quercus brantii*) was evaluated on few gram negative enteric bacilli and compared with some in-use antibiotics.

Duration and place of study: Nine months from October 2001 to July 2002 at the schools of Medicine & pharmacy, Ahwaz Jondi Shapour University of Medical Sciences, Ahwaz, Iran

Materials & Methods: Cold maceration with 70% methanol was used for extraction of seed hulls and different concentrations were prepared from the concentrated extract. The antimicrobial activity of the extract was examined using the standard MIC and disc diffusion method on *E. coli*, *Salmonella typhimurium*, *Shigella flexneri* and *Proteus mirabilis* and the activity was compared with those of gentamycin, nalidixic acid and co-trimoxazole in the next step.

Results: The antibacterial effect of the methanol extract on *P. mirabilis* and *E. coli* was significant and was directly concentration-related but had no significant effect on *S. flexneri*. Some concentrations had a similar or even better effect compared with nalidixic acid or co-trimoxazole, while the effect of 80% extract was not significant in general, except for *S. typhimurium* where its effect was equivalent to that of 25µg co-trimoxazole.

Conclusions: Although oak seed hull has some antibacterial activity apparently its anti-diarrheal effect is due to water absorption and protein precipitation in the body.

KEY WORDS: Seed hull, Antibacterial activity, *Quercus brantii*, *Salmonella*, *Shigella*, *E. coli*.

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INTRODUCTION

Infectious diseases account for about half of the deaths in tropical countries.¹ Progress in antimicrobial drugs, has introduced many antibiotics most of which are nontoxic, though all have side effects.^{2,3} Besides, incidents of epidemics due to drug resistant microorganisms

pose enormous public health concerns.⁴⁻⁶ So despite significant value of antibiotics, the increase of bacterial resistance has restricted their clinical application.⁷⁻⁹

Historically, plants have provided a good source of anti-infective agents and many of them remain highly effective in the fight against microbial infections. Besides, they are cost-effective and have fewer side effects.^{10,11}

Oak plant is a predominant genus in northern and central Iran and comprises many species. *Quercus* which grows in the central forest area of the country is one of the most important oak genera with 45 species; the predominant species being *Quercus brantii*.¹² The seed hulls of the plant have been used as anti-diarrheal in traditional medicine.¹³ Tannin is one of the major components of *Q. brantii* with contractive and disinfective

1. Dr. Azar D. Khosravi,
Department of Microbiology,
School of Medicine & Infectious and
Tropical Diseases Research Center.
 2. Dr. Atoosa Behzadi,
School of Pharmacy.
- 1-2: Ahwaz Jondi Shapour University of Medical Sciences,
Ahwaz, IRAN.

Correspondence:

Dr. Azar D. Khosravi
E-Mail: khosraviaz@yahoo.com

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effects.¹³ The aim of the present study was to investigate the antimicrobial property of seed hull of the plant and compare its effects with some in-use antibiotics.

MATERIALS AND METHODS

Extraction of oak seed hull: The fruit of the plant was collected from the Bakhtiari area (central Iran) during October to November 2001, washed and dried in air and seed hulls separated and macerated in 400 ml methanol (70% v/v) for 3 days.¹⁴ The mixture was then filtered and concentrated at 37°C.

Preparation of discs containing extracts: Different concentrations of 224, 168, 84 and 2.8 mg/ml (80, 60, 30 and 1% respectively) were prepared from the methanol extract. The concentrations were incorporated into sterile blank paper discs (Padtan Teb Inc., Tehran, Iran) and were dried at 37°C. The paper discs were weighed carefully for confirming exact amount of the extract being incorporated (compared to pre-weighed blank discs).

Preparation of bacterial suspensions: *Escherichia coli*, *Salmonella typhimurium*, *Shigella flexneri* and *Proteus mirabilis* which were isolated from stool specimens in the clinic were identified according to routine cultural properties and biochemical tests.¹⁵ Five strains of each were included in the study. A few colonies from the overnight culture of Eosin Methylene Blue (EMB) agar were transferred into approximately 4-5ml Trypticase soy broth (TSB) medium. The broth was incubated at 37°C for 3-4 hours and the turbidity of suspension was adjusted to that of a 0.5 McFarland barium sulfate standard. The standard suspension was used for both qualitative and quantitative antimicrobial assays. Merck Germany culture media was used in this study.

Susceptibility testing: The Agar disc diffusion test and tube dilution test for investigation of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) were carried out according to the method of Bauer and Kirby under strict adherence to NCCLS criteria.^{16,17} Briefly, in disc diffusion method, Muller Hinton (MH) agar medium

was inoculated with standard bacterial suspension and discs with different concentrations of the extract were placed in appropriate positions in the plate. The plate was incubated at 37°C for 24 hours; the inhibition zones of bacterial growth around the discs were measured and recorded. A similar qualitative assay was used to determine the susceptibility patterns of selected in-use antibiotics i.e. Gentamicin, Nalidixic acid and Co-trimoxazole and the results of both were compared. In the tube dilution assay, standard bacterial suspension was added to tubes containing 1ml broth and concentration of the extracts. The tubes were incubated at 37°C for 24 hours. The first tube in the series with no sign of visible growth was reported as the MIC. By culturing the tube on MH medium and subsequent incubation, no growth was assumed as the MBC of the extract.

All the tests were repeated 3 times for precise results and all the three repeat results showed no significant differences. SPSS software and T test were used for data interpretation included calculation of the mean values, standard deviation and investigation of significant differences in results.

RESULTS

In tube dilution method for determining MIC and MBC, the 80, 60 and 30% dilutions were excluded from the study because of formation of milky precipitation with the culture media, and 1% dilution showed only bacteriostatic effect on all tested bacterial strains with no bactericidal activity.

The results of disc diffusion method showed that the 80% concentration of the extract had significant effects on *E. coli* and *P. mirabilis* ($P < 0.05$ and $P < 0.01$ respectively) but it was not seen with 30 and 60% extracts (Fig-1). The inhibitory effect of 60% and 80% concentrations of the extract was significant for *S. typhimurium* ($P < 0.01$). None of the concentrations of the extract had significant effect on *Sh. flexneri*.

Antibiotic susceptibility testing for gentamicin, nalidixic acid and co-trimaxazole showed

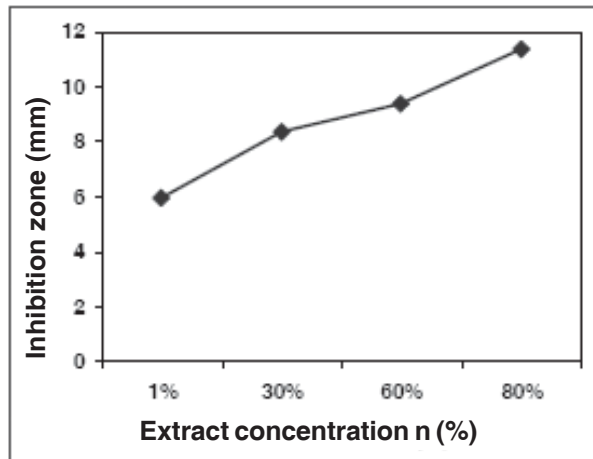


Fig-1: Comparison of inhibition zone diameter in *E. coli* using 0.1ml of different concentrations of extract in disc diffusion method.

high resistance of test bacteria compared to the standard strain which were sensitive to the drugs with *P. mirabilis* the effect of gentamicin compared to 80, 60 and 30% extracts was significant (Fig-2). In *S. typhimurium* the inhibition zone for gentamicin compared to 80% concentration of the extract was significant. The inhibition zone for gentamicin and co-trimoxazole compared with 60% and 30% extracts were also significant (Fig-3). None of the antibiotics were effective against *Sh. flexneri* or *E. coli*.

DISCUSSION

Antibacterial resistance especially among gram-negative bacteria is an important issue that has created a number of problems in treatment of infectious diseases and necessitates the search for alternative drugs or natural anti-bacterials.⁴

The present study indicated that the effect of the Oak seed extract on *E. coli* and *P. mirabilis* was concentration-dependent but not for *Sh. flexneri* or *S. typhimurium*. Since the condition of evaluation for extract effectiveness was similar for all the bacteria used, the difference in bacterial response was possible due to the nature of the latter bacterial species.

Despite previous reports on other properties of the oak seed hull,¹² our study was the first one on the antimicrobial effect of the plant in

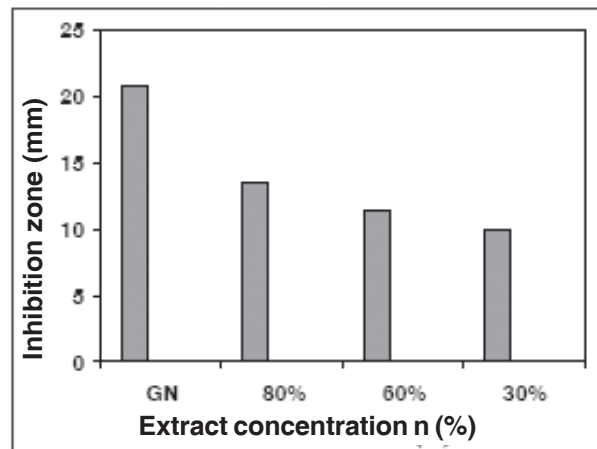


Fig-2: Comparison of inhibition zone diameter produced by gentamicin with different concentrations of extract in disc diffusion method for proteus.

this geographical region. However, there are numerous reports regarding antimicrobial effects of other plants in general, most of which involved the Labiatae family. Panizze and his colleagues (1993) investigated the effect of essential oil of four plants belonging to Labiatae family on some gram-negative bacteria including those used in this study. Their findings were similar to our results from antibacterial point of view.¹⁸ Larrondo and his colleagues in a similar survey using Labiatae plants concluded that the plants under investigation showed anti-bacterial and anti-fungal activities.¹⁹

The bacteria showed unexpected resistance to tested antibiotics which indicates the

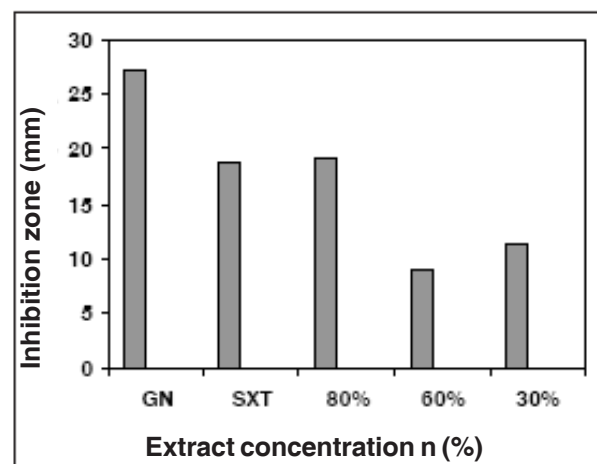


Fig-3: Comparison of inhibition zone diameter produced by gentamicin and co-trimoxazole with different concentrations of extract in disc diffusion method for salmonella.

failure of current chemotherapy against such bacterial infections requiring more effective antibiotics such as third generation cephalosporins.²⁰

Some concentrations of the extract were found to have a similar or even better effect compared with tested antibiotics. Based on previous studies on active constituents of oak seed hull,¹² Tannin is the most abundant compound in the plant whose major effect is anti-diarrheal because of water absorption and protein precipitation.²¹ The present study showed that it has some antibacterial activity as well. This was an *in vitro* investigation without interfering the body physical factors (such as gastrointestinal movements) and chemical effects (stomach enzymes and acid, mucous, etc.), however the response in the body might be quite different due to intervention of these natural factors. Further studies are needed for the clarification of the precise *in vitro* and *in vivo* antimicrobial activities of the plant extracts.

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