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Comparison of the Inhibitory Effects of Antibiotics With That of Pistachio Skin Extract on Enteric Bacteria

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Background: In spite of many advantages of modern medicine in comparison with traditional treatments, chemical therapeutic agents have side effects. Pistachio skin extract has antibacterial effects, but side effect has not been reported.
Objectives: The aim of this study was to compare the effects of pistachio skin extract with that of antibiotics such as amoxicillin, tetracycline, cefixime and ciprofloxacin, on enteric bacteria collected from patients with gastroenteritis.
Patients and Methods: Experimental method was used for this study. One hundred samples were taken from patients with gastroenteritis; then, the samples were characterized and the diagnosis was made. The pistachios internal soft skin extracts were obtained, using the decoction method and water as the solvent. Afterwards, the solution was crystallized and placed on 37°C and its water has been evaporated. The solution required two days for the entire water loss process. Antibacterial effect of the extract was analyzed with the disc diffusion method using Mueller-Hinton agar medium. After steeping the blank disc in the extract, bacteria reached 0.5 McFarland density, were cultured on plates with four antibiotic discs including amoxicillin, tetracycline, cefixime and ciprofloxacin. Next, depending on the type of bacteria, the inhibition zones were compared to determine the antibiotic inhibitory effects. Statistical analysis for comparing the antibiotics and pistachio skin extract effects was performed using chi-square tests.
Results: *Shigella* with 19% sensitivity and 1% resistance had the highest sensitivity and the lowest resistance to each of the antibiotics, including tetracycline, amoxicillin and ciprofloxacin; this difference was statistically significant ($P < 0.05$). The results showed that *Escherichia coli* with 13.6 mL sensitivity and 6.4 mL resistancy was the bacteria, for which antibiotics were least effective (amoxicillin: $P = 0.000$, tetracycline: $P = 0.049$, cefixime: $P = 0.000$); this finding was statistically significant ($P < 0.05$).
Conclusions: Regarding the natural therapeutic effects of pistachio skin extract and obligations of the routine antibiotics such as resistancy, using pistachio extract as a co-therapeutical agent with ordinary antibiotics can enhance the success rate of the treatment.

Keywords: Enteric Bacteria; Pistachio Skin Extract; Antibiotics

1. Background

One of the main concerns for patients with intestine infections is resistance of pathogenic bacteria to common antibiotics. The resistance rate in microorganisms such as *Shigella* has become a serious problem.

Currently, a wide range of antibiotics such as ciprofloxacin are very useful for treatment of these patients; however, this resistance can lead to various issues including treatment failure. Since most chemical medications have side effects, herbal treatments are recommended in different societies. One of these herbs that is used in traditional medicine is pistachio (1).

The pistachio stem extract is used digestion diseases treatment, which has been more effective than chemical medicines (2). Pistachio contains anacardic acid, which has antibacterial effects on methicillin-resistant bacteria. Anacardic acid prevents superoxide production, as well as lipoxygenase and xanthine oxidase radicals. It also has antibacterial effects against *Helicobacter pylori* and dental plaque causing bacteria (3-6), as well as anti-inflammatory, anti-wrinkle, and many other effects.

2. Objectives

The aim of this study was to evaluate the antimicrobial effects of pistachio extract and compare them with effects of four antibiotics on *Shigella* and *Salmonella* species and *Escherichia coli*.

3. Patients and Methods

This experimental study was performed at the end of summer of 2013 during the time of pistachio harvest. Fresh pistachios were placed in the shade and exposed

Implication for health policy/practice/research/medical education:
According to result of this survey and inhance resistance pattern to antibiotics therapy which has economical side for health services, this extract can substitute as a effective component for treatment.

to artificial wind; in the next step, their green skins were gathered, ground and boiled to obtain a fresh extract. Patients with gastroenteritis caused by enteric bacteria were selected. Before any treatment, stool samples were taken from the patients and 40 g of each sample was cultured on MacConkey agar, eosin methylene blue (EMB) agar, *Sallmonela* and *Shigella* (SS) agar, and deoxycholate agar with 24 hours incubation. For diagnosis, we used differential media and found the main cause of gastroenteritis. In every step, the incubated samples were analyzed by Gram staining to confirm the diagnosis. After all *Salmonella*, *Shigella* and *E. coli* samples were collected and diagnosed, they were divided to four groups. The antibiogram susceptibility testing or disc diffusion assay was performed on Mueller-Hinton agar (Merck, Germany) plates, which was already coated with bacterial suspension reaching 0.5 McFarland standard density. For group A amoxicillin (10 μg), group B tetracycline (30 μg), group C cefixime (30 μg), and group D ciprofloxacin (30 μg) were used (Merck, Germany). For all the groups, we also used blank discs steeped in pistachio skin extract without dilutation for comparing the antibacterial effect of the extract with that of antibiotics. Then inhibitory zone of the bacteria around each antibiotics disc was compared with that of pistachio extract.

The second method used was the minimum inhibitory concentration test (MIC). A pure culture of one microorganism is grown in Mueller-Hinton broth or other broths, as appropriate. Afterwards, the culture is standardized using standard microbiological techniques to obtain a concentration around 1000000 cells/mL. The more standard the microbial culture, the more reproducible the test results. The antimicrobial agent is diluted a number of times, usually to obtain a 1:1 ratio, through a sterile diluent (typically Mueller-Hinton broth). After that, a volume of the standardized inoculum equal to the volume of the diluted antimicrobial agent is added to each dilution vessel, bringing the microbial concentration to approximately 500000 cells/mL. The inoculated, serially diluted antimicrobial agent is incubated at an appropriate temperature for the test organism, for a preset period, usually 18 hours. The more consistent the incubation period, the more reproducible the test results.

After the incubation, the series of dilution vessels are observed for microbial growth, usually indicated by turbidity and/or pellets of microorganisms in the bottom of the vessels. The last tube in the dilution series that does not show any growth corresponds to the minimum inhibitory concentration (MIC) of the antimicrobial agent. Lack of growth was objected using spectrophotometer in OD 625 nm.

4. Results

Shigella, *Salmonella*, and *E. coli* samples obtained from infected patients were examined for susceptibly to all antibiotics and pistachio skin extract, by disk diffusion and MIC methods and the test results are listed below:

4.1. Shigella Samples Test Results

The resistance rate of *Shigella* to tetracycline was 24%, to amoxicillin 19%, to ciprofloxacin 14.5%, to cefixime 10%, and to pistachio extract 33.5%. The difference between effect of the pistachio extract and antibiotics on *Shigella* was tested using chi-square test and a significant difference was indicated ($P < 0.05$).

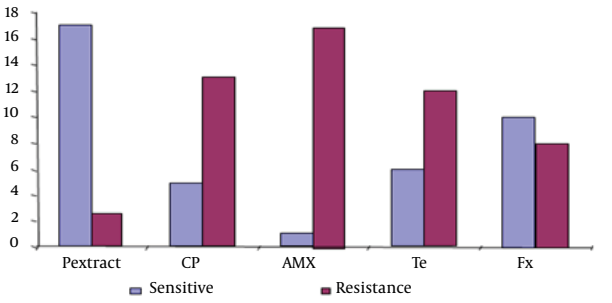

Figure 1. Survey of Effect of Pistachio Skin Extract and Ciprofloxacin, Tetracycline, Amoxicilline and Cefexime on *sheigella*

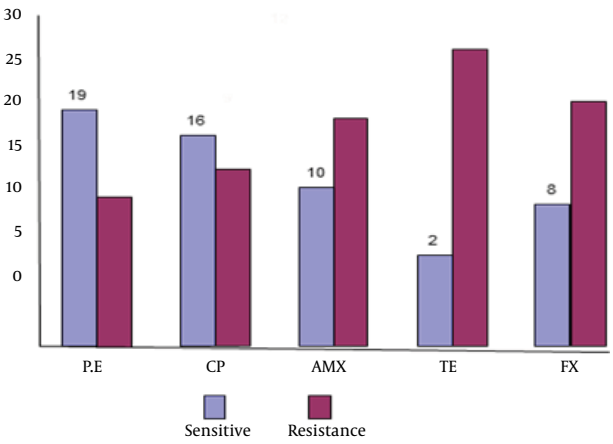

Figure 2. Survey of Effect of Pistachio Skin Extract and Ciprofloxacin, Tetracycline, Amoxicilline and Cefexime on E. coli

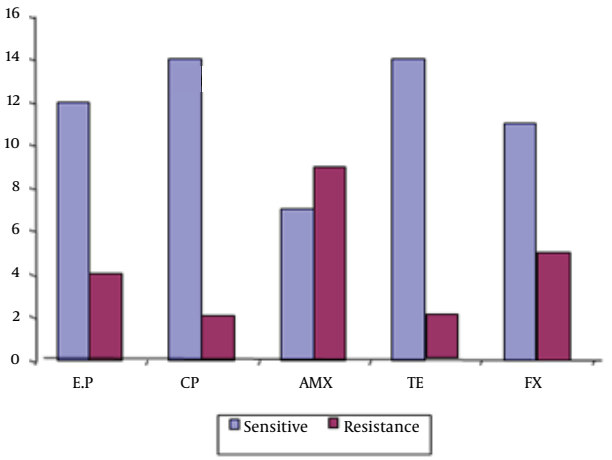

Figure 3. Survey of Effect of Pistachio Skin Extract and Ciprofloxacin, Tetracycline, Amoxicilline and Cefexime on *Salmonella*

Table 1. Comparison of Antibacterial Effect of Pistachio Skin Extract and Common Antibiotics on *Shigella*

Valid Case, No.	Nominal-Nominal Association, Contingency Coefficient	P value
90	0.519	0.000

Table 2. Comparison of Antibacterial Effect of Pistachio Skin Extract and Common Antibiotics on *E. coli* [a]

Valid Case, No.	Nominal-Nominal Association, Contingency Coefficient	P value
140	0.402	0.000

[a] Comparison between antibiotics and the pistachio skin extract and analysis of the level of sensitivity and resistance of *E. coli* bacteria to these two groups showed average response E coli to both group.

Table 3. Comparison of Antibacterial Effect of Pistachio Skin Extract and Common Antibiotics on *Sallmonella*

Valid Case, No.	Nominal-Nominal Association, Contingency Coefficient	P value
80	0.339	0.034

4.2. E. coli Samples Test Results

The resistance rate of *E. coli* to tetracycline was 13%, to amoxicillin 18.5%, to ciprofloxacin 8.5%, and to cefixime 14.5%, while resistance to pistachio skin extract was 11% (Figures 1 and 2). Chi-square test was used to compare the difference between the effect of each antibiotic and pistachio extract, which demonstrated a significant difference between the effects of amoxicillin, tetracycline and cefixime and the extract ($P < 0.05$), but the difference between the effects of ciprofloxacin and the extract was not significant ($P > 0.05$) (Tables 1 and 2).

4.3. Salmonella Samples Test Results

The resistance rate of *Salmonella* to tetracycline was 6.5%, to amoxicillin 11.5%, to ciprofloxacin 2.5%, and to pistachio extract 19% (Figure 3). The effects of each of the antibiotics and the extract were compared by the chi-square test, revealing significant difference between the effect of the extract and amoxicillin and cefixime ($P < 0.05$); but for ciprofloxacin and tetracycline, this difference was not meaningful ($P > 0.05$) (Table 3).

Compare to other bacteria, *Salmonella* was more sensitive to the pistachio skin extract and more resistant to amoxicillin than other agents used in this test.

5. Discussion

This assay as well as of similar studies results have proved the medical importance of traditional medicines and its necessity. A study was conducted in Algeria in 2010 on the oil extract of pistachio gum and its effect on Gram-positive bacteria like *Staphylococci* and Gram-negative bacteria like *E. coli* (2). This study was carried out using disc diffusion and MIC methods, proving that the antibacterial effect of this oil extract on Gram-negative and Gram-positive bacteria was more than other synthetic antibacterial agents. In our study, the pistachio skin extract had a strong antibacterial effect on *E. coli*; a finding similar to that of the Algerian study. The antibacterial effect of the pistachio skin extract in comparison to tetracycline, amoxicillin, and cefixime was significant, but ciprofloxacine didn't show significant antibacterial effect in this research . In a study performed during 2007-2008 in Iran, the antibacterial effects of the gum and essence of pistachio were examined on six bacteria including *Pseudomonas aeruginosa*, *Enterococcus feacalis*, *Klebsiella pneumonia* and *Bacillus cereus*, using disc diffusion and MIC methods. Standard samples of *E. coli*, *K. pneumonia* and *B. cereus* had the highest sensitivity and *P. aeruginosa* and *E. feacalis* had average sensitivity to the essence of pistachio, while *Staphylococcus aurous* had the lowest sensitivity to it. Gum of pistachio had no considerable antibacterial effect in comparison with its essence (2). In our research, all *E. coli* samples were collected from patients. The effect of the extract was considerably more than the effect of antibiotics.

During 2004-2005, a project conducted at the Shahid Beheshti University of Medical Sciences, Iran, showed that there were differences in the antibacterial and antifungal effects of pistachio fruit skin, depending on the type of extract; the aquatic extract had considerable effects on *S. aurous* and no effect on other microorganisms such as *Micrococcus luteus*, *E. coli*, *Salmonella* and fungi (7). Examination of the effect of alcoholic extract on *E. coli*, *S. typhi* type B, C, and *paratyphi*, proved that the extract had antibacterial effects on *Salmonella*, which was comparable with synthetic antibiotics such as amoxicillin and cefixime; yet, in comparison with ciprofloxacin and tetracycline, there was no meaningful difference. This difference can be due to different geographic plantation strategies of pistachio in Iran.

Another research, conducted at the research center of Ilam University, Iran, during 2011, investigated the effects of three types of pistachio extracts on *E. coli*, *S. aurous* and *S. epidermidis* and compared these effects to those of common antibiotics. The results determined that the extract had a greater effect on *E. coli*, *S. aurous* and S. *epidermidis* in comparison with regular antibiotics (4). Regarding a previous research on the *Enterobacteriaceae* family, causing digestive system infection, we can conclude that the extract has more effect on *Shigella* compared to the bacteria used in this study such as *Salmonella* and *E. coli*.

Finally, the effect of the extract at its lowest dilution on the microorganisms that infect the digestive system was comparable with the best synthetic antibiotics. Therefore, due to advantages such as less side effects as well as no need for pistachio import due to its domestic plantation, administration of this extract at least as a co-treatment factor can be useful. It is suggested to generate efficient amounts of this extract, which can lead to better

results. In addition, the effect of this extract can be observed through *in vivo* tests.

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