

Antibacterial Effect of Hydro-extract of *Pistacia atlantica* on Bacteria in *In vitro*

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ABSTRACT

Antibacterial resistant microorganisms as agents which cause infection diseases are fourth cause of mortality throughout the world, therefore there is a huge attempt to deal with this issue. New antimicrobial agents design based on high bactericidal potential, high specificity and low cross-reaction. Herbal remedy as an ancient treatment is considerable. Previously, herbal extract were exploited to treat infections such as; *Aloa vera*, *Thymus vulgaris*, *Allium sativum*. This study is aimed to investigate the effect of *Pistacia atlantica* on bacteria and compare its effect with routine antibiotics. Sample size was determined 30 numbers per each bacteria by STATCALC software (EPI-Info). Hydro-extract prepared from *Pistacia atlantica* that collected from Zagros mountains. Disk diffusion and well embedding method recruited to investigate antibacterial effect. MIC test done for hydro-extract and antibiotics. Statistic analysis done by Square Chi test, t independent test, t-test and fisher test. MIC of *Pistacia atlantica* extract were 163µg/ml, 104.16µg/ml and 204.67µg/ml for *E.coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively. These funding shown inhibition effect of this herbal extract but it indicate no affect on *H.pylori*. Funding indicate inhibit effect of hydro-extract on recruited bacteria except *H.pylori* thereby, it is suggested that phenol/chloroform-extraction could be have inhibit affect on *H.pylori*.

Key words: *H.pylori*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E.coli*, *Pistacia atlantica*, Hydro-extraction.

INTRODUCTION

Background

Herbal remedy as an ancient cure has been exploited for centuries. Recently, nearly to 1/3 of industrial drugs produced from herbal sources or modified after extraction¹. During last forty years scientists have been had huge effort to introduce novel antibiotics which have appropriate bactericidal effect and low toxicity therefore more than thousand ones had produced². Approximately 75% of world

population administrate herbal remedy to deal with complaints³. Hospital acquired infection could be very slight poisoning or life minatory infection^{4,5}. *Pseudomonas aeruginosa* known as a minatory factor of life specially among patients with burn wound and Cystic fibrosis patients. Prevalence of *H.pylori* among adults in developing countries arise up to 80% that is contributed to gastrointestinal disorders such as Gastritis, Peptic ulcer and gastric adenocarcinoma⁶⁻⁹. In the western of Iran, native people use herbal drugs to treat superficial, interior

and deep infections. *Pistacia antalica* belongs to *Anacardiaceae* and as native herb used for herbal remedy. The aim of current study focused on the effect of hydro-extract of *Pistacia atlantica* on bacteria¹⁰⁻¹².

MATERIAL AND METHOD

Extraction

Pistacia atlantica collected from Zagros mountains and after cleaning, drying and powdering. 1mg of powder added to 10ml DW and boiled for 15minutes then paper filter was used to filtration. Finally, water bath used to prepare 30°C for elimination process.

Sampling

30 clinical isolates for each bacteria isolated from hospitals and health publics of Ilam. Biochemical differential test exploited to determine bacteria. Biopsy samples cultured on Columbia agar with selective supplement at presence of CO₂. 48hours culture of *H.pylori* prepared in peptone water. Antibiogram test done based on Kirby-bauer for all strains, Muller Hinton agar with 10% Sheep Blood used to antibiotic susceptibility test of *H.pylori*. Serial dilution of extract added to embedded well on media and also routine antibiotic disk used. Culture incubated 3 days at 37 in 5%-7% Co₂.

Antimicrobial Susceptibility determination

Minimum Inhibition Concentration (MIC) for all of four isolates done based on E-test for 10 antibiotics. 0.5 µF suspension of each isolates

prepared and cultured on Muller Hinton agar then E-test strips laid on media. Diameter of no growth zone evaluated after 18 hours incubation at 35. Standards of *Clinical and Laboratory Standards Institute (CLSI)* were used to determine susceptibility. E-test is a special strip that saturated by serial dilution of certain antibiotic. In this test bacteria cultured on media same as disk diffusion method and strips are laid on the media. Susceptible strain shown an oval shape zone of no growth that can be observed by naked eyes. Confluence of this oval shaped zone with strip indicates MIC.

To determine MIC of extract, 0.5 µF suspension of bacteria added to TSB and serial dilution of extract then MIC read after 16-20 hours incubation. Inhibition effect of extract evaluated by agar diffusion method. Wells embedded on Muller Hinton agar and serial dilutions of extract pour into them after 20 hours incubation at 35, zone of no growth evaluated. To analysis obtained data of MIC and E-test, Square Chi test, t independent test, t-test and fisher test done.

RESULTS

Antibiogram result of *E.coli* indicates the lowest resistant to *Imipenem* whereas the highest resistant is shown to *Amoxicillin* by 83.3%. *Pistacia atlantica* extract shown inhibition effect against *E.coli* growth by MIC: 163µg/ml (Fig.1). Hydro-extract of *Pistacia atlantica* dose not indicate any effect on *H.pylori*.

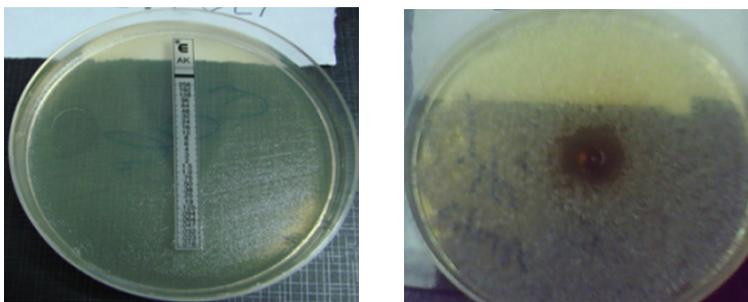


Fig.1: *E.coli*; E-test and well embedding test of *Pistacia atlantica*

The lowest resistant were to *Mropenem* and *Amikacin* while *Pseudomonas aeruginosa* has highest resistant for *Trimethoprim/Sulfamethoxazole* (TMP/SMX) by 6.6%. *Pastacia atlantica* extract has

MIC: 104.16 µg/ml for *Paeruginosa*. Diameter of no growth zone is investigated by various dilution that highest zone was 20mm by 100µl/ml of extract (Fig.2).

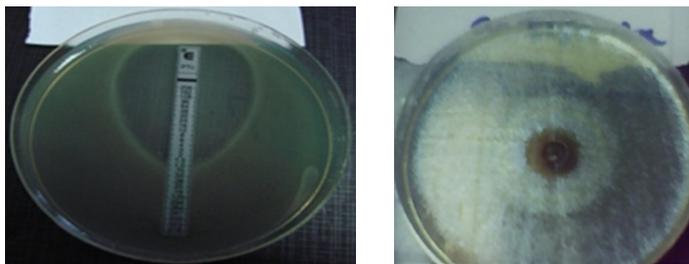


Fig. 2: *P.aeruginosa*; E-test and well embedding test of *Pistacia atlantica*

Staphylococcus aureus shown lowest and highest resistant to Vancomycin, Amoxicillin and Tetracycline (36.7%), respectively.



Fig. 3: *S.aureus*; E-test and Well embedding test of *Pistacia atlantica*

DISCUSSION

Salehi *et al* indicate the effect of methanol-extract of *Pistacia vera* on *E.coli*. Obtained MIC of methanol-extract is higher than hydro-extract that demonstrate better efficiency of hydro-extract than methanol-extract¹³. Masherghi *et al* shown that *Rosmarinus* extract have better efficiency than *Pistacia atlantica* extract on *E.coli* while Alcoholic extract of *hypericum perforatum*, *carthamus tinctorius*

dose not have effect on it¹². Torabi *et al* funding demonstrate that essence of *Eucalyptus kingsmillii* and *Eucalyptus salubris* have less inhibitory effect than *Pistacia atlantica* extract on *E.coli*.(10)

Ramezani *et al* prove inhibitory potential of *Pistacia atlantica* extract for *H.pylori* by MIC: 1.55 mg/ml¹¹. Nakhaie *et al* indicate the methanol-extract of *Citrus sinensis* and both aqueous and methanolic extract of *Cuminum cyminum* and *Artemisia dracuncululus* have strong affect on *H.pylori* growth and is better than *Pistacia atlantica* extract¹⁴.¹⁵. Current study shown that the hydro-extract of *Pistacia atlantica* fruit has inhibitory affect on *P.aeruginosa* by MIC:104/16 µg/ml and its peel extract has same potential. Among investigated bacteria, hydro-extract of *Pistacia atlantica* has more efficiency for *S.aureus* and regarding to comparison of antibacterial potential of this extract by routine antibiotics indicates that Cotrimoxazole has more inhibitory effect than studied extract on *S.aureus*. Hence, according to Salehi and Ramezani studies there is need to more investigate specially on methanol-extract or other kind of extract of this herb¹¹.

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