Pimpinella Anisum Extracts, Safe Anti-Bacterial Treatment

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Research

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Abstract

Introduction: Now a day, antibiotic-resistant bacteria are increasing and became a medical worldwide problem, therefore the achievement of a new safe disinfectant is deemed necessary. *Pimpinella anisum* (Pa) is an aromatic plant, belonging to the Umbelliferae family. It is a well-known traditional medicinal herb that has been used in old medicine as an antibacterial, anticancer, antiulcer, therefore, the aim of this study is evaluation of the anti-bacterial effect of PA extracts against Probiotics and pathogens bacteria.

Method: 96-well microplates MICs were determined by the broth microdilution method. Five Serial dilutions from 50 to 1 µg/mL concentrations were admitted for all bacteria which include: *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923, and Probiotics complex.

Results: PA watery extract demonstrates a statically inhibitory effect in just 50 g/L concentration against *E.Coli*, *E. Faecalis*, and Probiotic $p=0.000$ for all, whereas this concentration was 25 g/L for *Staph. Aureus* and Pu $p=0.000$ and $p=0.007$ respectively. PA alcohol extract displays the same effect in 1g/L for all bacteria $p=0.000$ for all bacteria.

Conclusion: Our results showed that PA in both watery and Alcohol extractions can inhibit both pathogenic and non-pathogen bacteria whereas active compounds are alcohol soluble. Long-time consumption of PA in an herbal product could disrupt normal bacteria of the gastrointestinal system while PA watery extract can promote Probiotics.

Introduction:

During the new decades, the communities of the extent resistance microorganism to conventional antibiotics are increased [1]. This adverse fact led to creating multidrug-resistant bacteria, and encounter side effects of synthetic antibiotics consumption, therefore novel bactericidal product achievement is apparent essential [2]. Different studies described the antibacterial properties of *Pimpinella anisum*.

Pimpinella anisum (Pa) is an aromatic plant, belonging to the Umbelliferae family [3, 4]. Anise plant ranges 30–70 cm altitude which has ternate pinnate simple leaves[4]. The biological and pharmacological investigation has shown that some *Pimpinella* species contain differentials properties such as estrogenic, anti-inflammatory, anticonvulsant, antispasmodic, antioxidant, antimicrobial, antifungal, insecticidal, and repellent activity [5–8].

It is a well-known traditional medicinal herb that has been used in old medicine as an antibacterial, anticancer, antiulcer, and as muscle relaxant [9]. Moreover, *Pimpinella* extract has favorable effects on menopausal hot flashes and dysmenorrhea in women It can also reduce morphine dependence [4, 9–11]. Furthermore, it is reported among diabetic patients, Pa could reduce the blood lipid and sugar level. Trans-anethole, γ -hymachalen, para-anisaldehyde, methyl chavicol, and estragole are the main chemical compound in Pa extract [12, 13]. *Pimpinella anisum*(PA) is a natural controlling agent against some

number of human and plant pathogens, and are also has potential healing properties and resistant activity to necrosis and cytotoxicity[3, 14–16]. The essential oil of PA is taken out from the aerial parts or root of the plant [14, 17]. The studies showed that the essential oil components of *Pimpinella anisum* gathering from the root part consisted of α -pinene, p-cymene, and limonene, whereas β -bisabolene, β -sesquiphellandrene and trans α –bergamotene are the main chemicals material which is extracted from the aerial part of PA[12, 14, 18] ().it seems the main PA active substance composition could be varied to different parts of the plant and also differences geographical country[12, 19](). Importantly, the use of antimicrobial compounds of the plant is not well known its side effects and may be associated with the removal of beneficial bacteria [8, 19–21]. One of the main beneficial bacteria groups is Probiotics [22] (). These are diver's non-pathogen bacteria which help for the treatment of acute diarrhea protect the intestinal organ from invasion and colonization, decrease pH of the intestine and regulate immune response[23–25](). Therefore, the aim of this study is evaluation of the anti-bacterial effect of PA extracts against Probiotics and pathogen bacteria.

Materials And Methods

Material and Method

- Plant collection

In March 2021 from the Northeast of Iran, herbal samples were arbitrarily collected and were recognized by naturalist botanists. The obtained samples were screen for specific *Pimpinella anisum* and then were naturally air-dried be stored in a low moisture place, protected from direct sunlight for about one week. After that dried plants were kept in plastic vials up to extraction time.

- Preparation of plant extract

The powder form of Plant materials was produced after dried and grind by the manual mechanical grinder. Planet powdered samples (50 g) were soaked with 500 mL of 99.8% ethanol (Merck, Germany, EC num: **200-578-6**) and were mixed continually be placed on a rotary shaker (Hitech, Germany) for 24 h at room temperature- protected from light. The same procedure was applied by distilled water for watery extract. Ethanol alcohol has been chosen as a solvent because of its use in form of oil for traditional medicines. Both Extracts were subsequently filtered. Rotary vacuum evaporator RV 8 V-C (IKA, Staufen Germany) was used for reconcentration of alcohol extract by low-level heating at 38-40°C. The dried powder was resolved in PBS buffer at the original concentration afterward were stored at 4°C until tested.

- Microorganisms and media

One of the most gram-positive cocci, three common entheogenic gram-negative bacterial and probiotics complex were collected in this study. Microorganism base on their American Type Culture Collection (ATCC) from Baharafshan Co-Iran purchased in the form of lyophilized strains, which contain:

Enterococcus faecalis ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923.

Probiotic was obtained from standard mixture formula. Femilacte® is a synbiotic combination (probiotic + prebiotic) suitable for all family members and contains high amounts (around 10^9 CFU) of nine beneficial and safe bacterial strains such as *Bifidobacterium lactis*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium bruh*, *Bifidobacterium langum*, *Lactobacillus rhamnosus*, *Streptococcus thermophilus*, *Bifidobacterium bifidum*, and *Lactobacillus Plantarum*, along with fructooligosaccharide as a Prebiotic. All strains and Probiotics used in this product include the mentioned microorganisms were recovered in Mueller-Hinton broth (MHB) (QUELAB, Montreal, Canada) at 37°C for 24h. Cultured bacteria adjusted for inoculum concentration by MHB, the turbidity of the microorganism suspension was obtained to a 0.5 McFarland standard (1.5×10^8 CFU/mL). Optical density was calculated by spectrophotometer Tajhizat Sanjesh (Esfahan-Iran) at 600nm.

- Minimum inhibitory concentration (MIC) assay

96-well microplates MICs were determined by the broth microdilution method. Five Serial dilutions (100 µL) of all herbal extract (Watery and Alcohol) were dispersed into the plate and diluted in the MHB provide ranging from 50 to 1 µg/mL concentrations. Thereafter, maintained microorganism suspensions were added to the microplates then were inoculated with the final concentration 5×10^5 CFU/mL for bacteria and Probiotics complex. Each dilution was examined triple for each bacterium. Plates were then incubated at 37°C for 24 h. Microorganism expansion was calculated in terms of turbidity recorded by OD measurements at 405 nm BioTek ELX 800 microplate reader, (Winooski, VT). The MIC was calculated as the lowest concentration that showed $\geq 80\%$ inhibition of microbial growth compared to extract-free growth control. All-optical densities were measured base on blank bacteria medium and herbal extracts. The herbal extract Added to MHB without bacteria suspension used as a negative control. The bacterial suspension with an equal volume of MHB was used as positive controls for microorganism growth. All data in this study were reported in the median/mode of MICs obtained from three independent experiments that were assayed in triplicate.

Statistical Analysis

Standard deviations and the mean values for all replicates were calculated by using SPSS version 16. Independent sample T-test and Mann-Whitney U test were used to compute the means differential values between groups. The significant statistical differences were considered p values < 0.05 .

Results:

PA watery extract results:

Normal test statically analysis was done for all bacterial growth values in five concentration groups of watery LA extractions. One-Sample Kolmogorov-Smirnov Test showed that all examined bacteria have a

normal distribution. Based on the Independent Samples Test, MIC microplate analysis data demonstrate that PA watery extract has an inhibitory effect on E.Coli, E. faecalis, and P. aeruginosa in just 50 g/L concentration by mean 0.195 ± 0.010 and 0.276 ± 0.023 $p = 0.000$, $p = 0.000$ and 0.380 ± 0.009 $p = 0.000$ respectively. Staphylococcus aureus growth got stopped in 25 g/L mean 0.277 ± 0.029 (graph 1) respectively.

Probiotics were not inhibited by this concentration whereas its growth accumulates, mean of control positive 0.244 ± 0.026 and 0.334 ± 0.016 for herbal extract $p = 0.000$. These data were shown in graph 2.

PA Alcohol extracts results:

All bacterial growth values in the five concentration groups of alcohol LA extractions were analyzed by Normal test One-Sample Kolmogorov-Smirnov, which showed that all included microorganisms have parametric growth values. Independent Samples Test for MIC microplate analysis data demonstrate that PA alcohol extract has an inhibitory effect on staphylococcus aureus, E.Coli, Enterococcus faecalis, and Probiotics in all concentration groups ($p = 0.000$, 0.000 , 0.000 , 0.000 , and 0.001 respectively). These data were shown in graph 3.

Conclusion:

Pimpinellaanisum (Pa) is an aromatic plant, belonging to the Umbelliferae family [26]. It is a well-known traditional medicinal herb that has been used in old medicine as an antibacterial, anticancer, antiulcer[27, 28]. As our data demonstrate above PA extract has a proper antibacterial activity, where 50 g/L concentration of PA watery extract can inhibit all examined bacteria while for staphylococcus aureus and P. aeruginosa this amount was 25 g/L. This data suggests that LA watery extract has dose depended on anti bacterial activity, which lower LA concentration have not stopped the bacterial growth or can prompt their division. This fact is observed for Probiotics bacteria whose PA watery extract promotes bacterial growth. The 1g/L concentration of PA alcohol extract displayed the same inhibitory activity, therefore, it seems PA alcohol extract has the strongest anti-bacterial effect than watery form. This data suggest that PA's main anti-bacterial compounds may be alcohol soluble. Furthermore, Probiotics as non-pathogenic microorganisms can inhibit such as pathogenic ones, therefore long-time consumption of PA in the herbal product could disrupt normal bacteria of the gastrointestinal system while watery extract in 50 g/L concentration accelerates beneficial bacteria growth and inhibit pathogen microorganism expansion.

Our results showed that PA in both watery and Alcohol extractions can inhibit both pathogenic and non-pathogen bacteria in 25–50 g/L concentration whereas 1 g/L of alcoholic extraction demonstrates the same effect.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Will be sent in future

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

This is the self fund study

Authors' contributions

MS: Is providing material and help to perform this study

AK: Is providing material and help to perform this study

ZF: Is analyzing data and provide the herbal extract

MMA: is performing, deigning and writing the article

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Figures

Figure 1

Staphylococcus aureus growth values in PA extract showed that just 25g/L concentration can significantly inhibit bacterial growth.

Figure 2

Probiotics growth values in PA extract showed that all concentrations can significantly induce bacterial growth.

Figure 3

All pathogen and Probiotics growth values in PA extract showed that at least 1g/L concentration can significantly inhibit bacterial expansion.