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Antibacterial Effect of Two Persian Traditional Natural Compounds - Anbar Nesara and Esfand-'S Fume on Treatment of the Bacterial Vaginitis

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Abstract

Background study: Drug resistance is one of the main problems in the treatment of infections, and coping with this phenomenon and the use of natural substitutes is very important. The use of medicinal fumes in the treatment of many diseases, including microbial diseases and infections in Iran, Turkey and Malaysia has long been popular. Among all of them, fume from burning the Female donkey Dung (Anbar-Nesara) and Peganum harmala or Esfand (in Persian) are much more famous. This study aimed to compare the antimicrobial properties of the smoke of Esfand's grains and smoke of Anbar-Nesara on Pseudomonas aeruginosa and Staphylococcus aureus and its effect on treatment of Bacterial vaginitis.

Materials and methods: In this experimental study, fume of Esfand and fume of Anbar-Nesara were considered as case and antibiotic and straw smoke were considered as control. Standard strains of Pseudomonas aeruginosa and Staphylococcus aureus were given in appropriate culture media. The discs of antibiograms smeared with Anbar-Nesara, Esfand and straw were placed separately on sterile pins on the surface of the plates containing these strains and after 48 hours incubation at 37°C for the presence or absence of growth halo of bacteria has been investigated. The smoke operation was repeated every 20 minutes and up to 24 times in the designed compartments.

Results: Both species were resistant to straw smoke. Staphylococcus aureus was susceptible to smoke of Anbar-Nesara and Esfand, and Pseudomonas aeruginosa was susceptible to smoke of Anbar-Nesara only. In the control group, Staphylococcus aureus was resistant to Cloxacillin, and Pseudomonas aeruginosa was only susceptible to erythromycin and ciprofloxacin. As the smoke time increased, the diameter of the non-growth zone increased in sensitive areas, in other words, the antimicrobial effects of smoke increased.

Conclusion: Considering the research findings and the antimicrobial effects of Anbar-Nesara smoke on highly resistant microbes such as Staphylococcus aureus and Pseudomonas aeruginosis, more extensive researches are needed on the active ingredients and properties of the Anbar-Nesara's smoke.

Keywords: Anbar-Nesara; Peganum harmala; Bacterial vaginitis

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Introduction

Bacterial vaginosis (BV) is a common vaginal disorder in women of reproductive age. Since the initial work of Leopoldo in 1953 and Gardner and Dukes in 1955, researchers have not been able to identify the causative etiologic agent of BV. There is increasing evidence, however, that BV occurs when *Lactobacillus spp.*, the predominant species in healthy vaginal flora, are replaced by

anaerobic bacteria, such as *Gardenella vaginalis*, *Mobiluncus curtisii*, *M. mulieris*, other anaerobic bacteria and/or *Mycoplasma hominis*. Worldwide, it estimated that 20-30% of women of reproductive age attending sexually transmitted infection (STI) clinics suffer from BV, and that its prevalence can be as high as 50-60% in high-risk populations (those who practice commercial sex work (CSW). Epidemiological data show that women are more likely to report BV if they: 1) have had a higher number of lifetime sexual partners; 2) are unmarried; 3) have engaged in their first intercourse at a younger age; 4) have engaged in CSW, and 5) practice regular douching. In the past decade, several studies have provided evidence on the contribution of sexual activity to BV. However, it is difficult to state that BV is a STI without being able to identify the etiologic agent. BV has also emerged as a public health problem due to its association with other STIs, including: human immunodeficiency virus (HIV), herpes simplex virus type 2 (HSV-2), *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG). The most recent evidence on the association between BV and CT/NG infection comes from two secondary analyses of cohort data conducted among women attending STI clinics. Based on these studies, women with BV had a 1.8 and 1.9-fold increased risk for NG and CT infection, respectively. Taken together, BV is likely a risk factor or at least an important contributor to subsequent NG or CT infection in high-risk women. Infectious diseases are one of the most commonly diagnosed diseases that have always been humanized and many efforts have been made to identify the causes, treatment and control of them. One of the most important causes of infection in humans, especially *Staphylococcus aureus* and *Pseudomonas aeruginosae* are nosocomial infections. Almost all people during their life are somewhat infected with *Staphylococcus aureus*, with symptoms ranging from mild to moderated mild infections and life-threatening infections. *Pseudomonas aeruginosa* is widely distributed and found in soil, water, plants and animals, and is the most important cause of infection in people with immune deficiency. *Pseudomonas* are gram negative, aerobic and moving bacilli that often form a small number of intestinal and intestinal flora, and opportunistic pathogen in patients with defective disorder. This pathogen is an opportunistic agent for infection, especially in patients with cystic fibrosis and burns. It is also considered as one of the most important factors in nosocomial infections. *Pseudomonas aeruginosa* is resistant to many antimicrobial agents and antibiotics, and in addition to its intrinsic resistance, it is resistant to drugs in many antibiotics during treatment. However, the emergence of multiple drug resistance strains in various hospitals is increasing, which is one of the most important problems in controlling infection in hospitals. On the other hand, the contamination of hospitals and treatment centers to pathogens is one of the most important issues that can lead to death of patients admitted to these centers. In addition, to exacerbating the illness and death of susceptible patients, increase hospitalization time and, as a result, increase in treatment costs, have a great impact on the economy. Among the causative agents of nosocomial infections, *Pseudomonas* bacteria are more important because of the ability to adapt well to the environment and can be present at any location in the hospital. In addition, due to an increase in antibiotic resistance, especially as a multi-drug, this organism has caused many problems in treating infections caused by them. Various drugs are used to treat infectious diseases such as Aminoglycosides, Cephalosporin, Vancomycin and the like, as well as medicinal plants such as Garlic and Thyme. The bacterial resistance to chemical antibiotics limits the ability of physicians to treat some infectious diseases that are often fatal. The annual mortality rate from hospital infections is the sole cause of forty thousand deaths in the United States, which is usually due to the increased bacterial resistance to antibiotics. Therefore, coping with drug resistance phenomenon seems to be of major importance. On the other hand, due to the importance of bacteria such as *Pseudomonas aeruginosa* in burn and surgery, and the vast resistance of this bacterium, many antibiotics and complications from their use, the use of natural substitutes has been considered more. However, the use of medicinal plants for the treatment of diseases has been using for centuries. Today, although a large proportion of consumable drugs are synthetic, it has been estimated that at least one third of all drug products or plant origin have been or have been transformed after extraction from the plant. Many of the old drugs are already used in the same old form, including in the form of smoke. In more than 50 countries, the use of conventional medicine is even more commonplace among adolescents. One of these medical smokes is smoke from the burning of harmel seed or Esfand (*Peganum harmala*) and Anbar-Nesara. It is believed that these smokes have more and faster therapeutic effects. Esfand is a traditional herb in Iran and has various uses in traditional medicine. Herbaceous Esfand is a split leaf, large and greenish flowers, whose fruit has a spherical capsule containing black beans, and the seeds have an alkaloid such as harmalin and harmonol. Harmalin has a therapeutic effect with

Table 1 Susceptibility pattern of *Staphylococcus aureus* and *Pseudomonas aeruginosa* compared to diffused discs with different materials and duration of smoke.

Type of Microbe	*Staphylococcus aureus*					*Pseudomonas aeruginosae*				
Time (Hour)	1	2	4	6	8	1	2	5	6	8
Smoke										
Sergin	Resistant	Resistant	Resistant	Sensitive	Sensitive	Resistant	Resistant	Resistant	Sensitive	Sensitive
(Dung)				15 mm*	21 mm				6 mm	10 mm
Spand	Resistant	Resistant	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant
(*Peganum harmala*)			4 mm	5 mm	14mm					
Straw	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant

toxic effects, Fungicides and bacteria. This plant belongs to family of *Zygophyllaceae* that in Iran called Esfand, and has long been considered as one of the most important medicinal plants. For this seed, the properties of hypothermic and halocinogen gene are reported. It is traditionally used as an aboriginal plant in Asia and Africa. Various pharmacological studies have been carried out for Esfand, including antimicrobial, anti-tumor, and inhibitor of monoamine oxidase activity. In Iran and Turkey, Esfand's smoke is widely used as an eyesore and an antiseptic agent. On the other hand, animal feces, or in the term "dung or Anbar-Nesara ", are materials in nature that have different uses. Animal stool has been used to treat some human diseases. For example, a newly born donkey's feces used to treat severe cough. The smoke from the burning of donkey's feces, including the medical application, is considered as an antimicrobial agent in some countries and is used as an antiseptic and antimicrobial agent. Due to the fact that these two substances are available and relatively inexpensive and easy to use. Since there is no scientific and systematic review of the efficacy and anti-microbial properties of Anbar-Nesara, a comparative study with Esfand has been designed in this study. In this regard, the present study was conducted to compare the antimicrobial properties of Esfand and Anbar-Nesara smoke against *Pseudomonas aeruginosa*, *Staphylococcus aureus in vitro.*

Analyzation method

The present study was an experimental-interventional study. In this study, antibiogram discs were used for fusion, so that different types of smoke and at different times in the designed compartment smelted antibiogram discs in the system. Disk containing antibiotics and straw were also considered as control groups. Esfand's smog and the smoke of Anbar-Nesara were considered as the case group. In this study, ATCC strains with the following characteristics were used. *Pseudomonas aeroginosa*: ATCC 27853, *Staphylococcus aureus*: ATCC 25923, *Pseudomonas aeruginosa* and *Staphylococcus aureus* colonies were prepared with a half-MacFarland turbidity equivalent suspension, and then, using a sterile swab along with a flame, each bacterium was cultured on a muller hinton agar, bladagar and EMB in a lawn. Then, discs (discs antibiograms) smeared with Anbar-Nesara or spindle were separately placed on the plate surface with sterile pins, and after 48 hours incubation at 37°C in terms of the presence or absence of a growth halo Reviewed and reported. In order to smoke the Blank antibiogram discs, special containers designed for this purpose were used, the materials were continuously burned, and smoke was introduced into this compartment. The smoke action was repeated up to 8 hours. Antibiotic discs were fused in this chamber for 8 hours and transferred to different culture media. It should be noted that for the purpose of this study, extracts of Anbar-Nesara has gotten from a female donkey that was stored and fed in a specific place were used. They were designed and burned after drying in a special compartment, and antibiogram discs were exposed to smoke. Every 30 minutes, 20 grams of Esfand, one slice of Anbar-Nesara weighing approximately 10 grams and the weight of the straw was burned in separate enclosures. In order to adjust the amount of smoke during different hours, this was done in three compartments containing blanc antibiogram. These experiments were performed to increase the accuracy of the study in three replications and the diameter of the non-growth halo zone was measured. In this research, after the culture of the bacteria, tests were carried out to determine the susceptibility or resistance of the smoked disks with the different materials (based on the presence or absence of the growth halo and the diameter of the halo). In this study, disks containing different antibiotics (Cloxacillin, Ciprofloxacin, Amikacin, Gentamicin, and Vancomycin) and smoked discs were used as control group. Data collected in these observations were analyzed by descriptive statistics [1-10].

Results

Halo of non-growth zone was formed around the discs smeared by smoke of Anbar-Nesara and Esfand in a culture medium containing Staphylococcus aureus. On the other hand, the increase in the duration of smoke time caused the increasing the diameter of the non-growth zone around the disks, and the highest non-growth halo was formed around the smoke disc provided with the Anbar-Nesara after 8 hours. The results of this study showed that in the culture medium containing *Pseudomonas aeruginosa,* the highest diameter of the non-growth zone was formed around the Anbar-Nesara smoked discs after 8 hours. Esfand's smoke in this regard was not effective on *Pseudomonas aeruginosa* strain. In this case, the increase in the time of smoke caused the formation of a non-growth zone **(Table 1).** In this study, *Staphylococcus aureus* was resistant to Cloxacillin and was sensitive to Ciprofloxacin with 34 mm non-growth zone halo, Amikacin with 27 mm, Gentamicin with 20 mm, and Vancomycin with a 19 mm non-growth zone halo. While *Pseudomonas aeruginosa* was resistant to Imipenem, Gentamicin, Nalidixic acid, and Nitrofurantoin, it was susceptible to erythromycin with a 10 mm non-growth zone halo and Ciprofloxacin with a 43 mm non-growth zone halo [11-13].

Discussion

The results of this study showed that the antimicrobial effects of smoke caused by burning of Esfand and Anbar-Nesara in the elimination of two standard strains of Staphylococcus aureus and *Pseudomonas aeruginosae* with different smoke duration were different. By increasing the time of smoke, the diameter of the non-growth zone increased. In other words, the antimicrobial effects of smoke increased by the time of smoke. After Smoke-feeding the discs by Anbar-Nesara and Esfand, the non-growth zone around these discs was formed in a culture medium containing *Staphylococcus aureus*. This finding is consistent with the findings of the study of Parvin that studying the effects of Esfand smoke on *Staphylococcus aureus*. Esfand smoke contains various compounds such as aromatic and phenolic derivatives, indole derivatives, alcohol and ketone compounds, normal alkalins, and fatty acid derivatives. Khorsandi and Moghanian [13] concluded that among the Esfand's alkaloids, only Harmilin is unaltered in smoke and the effects of this smoke are likely to be related to this substance. In the present study, increasing the duration of smoke in the groups of Anbar-Nesara and Esfand increased the diameter of the non-growth zone halo around the discs. This finding showed that in spite of drug resistance to Staphylococcus aureus, Esfand and Anbar-Nesara smoke can be effective in removing this important pathogenic microorganism.

Najafi [10] also concluded that Esfand smoke is effective only on gram-positive bacteria and that it is effective on gram-negative bacteria only at high doses (8 hours of smoke), which is consistent with the findings of this study. Particularly, the effect of smoke time on the elimination of pathogenic microorganisms is consistent. On the other hand, in a culture medium containing *Pseudomonas aeruginosa*, the non-growth zone was formed around the Anbar-Nesara smoked discs and did not form an non-growth zone halo around the Esfand smoke disc, even after 8 hours of blanching. What is known is that, the chemical composition of the smoke resulting from the burning of the Anbar-Nesara and the Esfand has different effects and different antimicrobial properties. However, it should not be forgotten that the diameter of the non-growth zone in various types of samples was influenced by other factors, such as the permeability of the antimicrobial agent that was in the agar medium and its molecular size. Donkey feces contains a large amount of organic matter and nitrogen and a large amount of plant material eaten by them. It is important to note that the substances used and the digestion of the food influence the concentration of toxic and essential substances in the compound. In fact, Anbar-Nesara can be a source of antibiotic and has miraculous properties. Miracle cure of the Anbar-Nesara is confirmed based on the observed experiences. On the other hand, Anbar-Nesara have different microflora and its substances can produce different antibiotics that are effective on different organisms. Old Egyptian medicine has been named stool smoke as a great flammable substance. This feature and the ability to burn due to its fat and fiber content. Studies have shown that the main components of the smoke caused by the burning of some dried plants include formic acid, acetic acid, butyric acid, caprylic acid, vanillic acid, sulfuric acid, dimethoxy phenol, gliuccal, furfural, methanol, ethanol, octanal, acetaldehyde, acetyldehyde, Acetone and 3 to 4 benzobarbin. The studies have shown that the smoke which caused by burning of them has more than 200 units. Many smoke compounds such as formaldehyde, stalled, methanol and some aromatic compounds such as phenols, gayocles, crossover have bactericidal or bacteriostatic property. The bacterial activity of smoke is related to its formaldehyde component. The effect of each of the above compounds is unique, but experiments have shown that, due to smoke, the effect of a combination of compounds is higher than effect of each one separately. On the other hand, many of the compounds in the smoke and their antimicrobial effects have not yet been identified. These characteristics relate to smoke from wood burning, while no studies have been done on the materials in the smog from smogs, and the possibility of justifying the mechanism and antimicrobial compounds of this smoke is currently absent. These effects can be attributed to the composition of food and plants which used by the animal. The present study showed that in many cases the smoke from burning animal feed does not have antimicrobial effects in the extent and magnitude of its smoke, which is probably The presence of other compounds in Anbar-Nesara may arise from interactions in the animal's digestive system and even probiotics and antibiotics in it. On the other hand, Sorouri studied the effects of spade smoke on *Pseudomonas aeruginosa, Staphylococcus epidermidis, Escherichia coli, Staphylococcus aureus*, *Aspergillus neigera*, which was effective on all these microorganisms. Differences in the effects of Esfand's fume may also be due to the nature of the examined microbes and the permeability of the antimicrobial agents of Esfand's fume in these pathogenic microbes. One of the materials in Anbar-Nesara Liginin is that its hydrolysis causes the formation of some compounds with inhibitory properties of microorganism's growth. Based on the origin and inhibitory effects, three major groups of weak acids, eruption compounds and phenolic compounds are formed due to this hydrolysis. Sunen also concluded that the antimicrobial effects of fume from burning wood on the dose and concentration of phenols present in the smoke have been associated with inhibitory effects on the growth of *Staphylococcus aureus, Listeria monocytogenes*, and *Yersinia enterocolitica* and on inactive *Salmonella antridis*. This finding is consistent with the findings of the present study. The cause of the antimicrobial effects of smoke is the phenolic-polar compounds in the liquid phase of smoke, which these phenolic compounds have high water solubility in the water, which increases the potential for contact and interaction with the target organisms and their fatal effects. On the other hand, the presence of acetic acid in smoke in the liquid phase can also be responsible for its antibacterial effects. The findings of this study are in part consistent with the present study. On the other hand, in this study, phenolic and polar compounds may be used to justify their antimicrobial effects. Fermentation and digestion process in the digestive system of the animal in question may cause some antibiotic agents in the animal's stool, which is one of the mechanisms for the antimicrobial effects of the Anbar-Nesara's fume. Heat generates the material in a plant or product into micronutrient and microscopic components and allows them to absorb more of these materials. This case refers to the mechanism of smoke infiltration in cells.

Conclusion

Regarding the antimicrobial effects of Anbar-Nesara and Esfand's fume, on highly resistant microbes such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are less susceptible to the drug, the probable medication value of these two compounds is determined. The results of this study for further studies on the antimicrobial properties of various types of medical smoke, such as smoke from burning animal products and medicinal plants, are suggested. In the future studies, it can be suggested to identify the active ingredients in various types of smoke. Medical products should be provided to allow the clinical and practical use of these natural and available products.

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