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Antibacterial effects of methanolic and aqueous extracts of eight plants in traditional medicine

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Abstract

Background and Objective: *Escherichia coli* bacteria often causes infections in the gastrointestinal tract and other parts such as the urinary tract. *Staphylococcus aureus* is a common pathogen that causes infections in the skin, upper respiratory tract, and many parts of the body. In recent years, resistance to these bacteria has become one of the medical problems of antibiotics. Flowering herbs from the past are used to treat genital and gastrointestinal tract infections, and traditional herbal remedies are used to treat prostatic inflammation, gastric and intestinal ulcers, and lungs, kidneys and bladder problems. Hypericum perforatum is used to relieve the cough, symptoms of common cold, sputum, and healing wounds and injuries. *Artemisia absinthium* is beneficial for the treatment of arthritis, and inflammation of the spleen and hepatitis and the lavender plant is antiseptic. The aim of this study was to determine the antimicrobial effect of the extracts of these plants.

Materials and Methods: In this study, the antimicrobial effects of aqueous and ethanolic extracts of the above-mentioned plants on standard bacteria were studied. Methanolic and aqueous extracts were performed using standard methods. Serial dilution of the extracts was prepared. The extracts were then concentrated in water bath. Determination of hypersensitivity was done by disc diffusion method for each extract and the findings were analyzed.

Results: The results of this study showed that the alcoholic and aqueous extracts of *Artemisia absinthium* on *Escherichia coli* and *Achilles millefolium* aqueous extract on *Staphylococcus aureus* have the highest effect. The antimicrobial effect of the aqueous extract of *Ruta graveolens* and alcoholic extract of *Artemisia absinthium* was more than the others.

Conclusion: The widespread use of antibiotics and drug resistance has led to more attention to medicinal plants. Some plants extracts have a significant antimicrobial effect. The plants studied in this study had an inhibitory effect on *Escherichia coli* and *Staphylococcus aureus* strains. It can be hoped that with further investigation of the various forms of the extracts, effective and safe drugs were obtained to control the bacteria.

Keywords: Escherichia coli, *Staphylococcus aureus*, Medicinal plants extracts, Antibacterial effects

1. Introduction

E. coli bacteria have a high physiological and metabolic convergence potential. These bacteria include many nonpathogenic species that coexist as a normal flora in the intestines of humans and animals. Pathogenic species cause intestinal infections in humans and animals. These bacteria are significantly able to survive at low pH and this is due to the presence of various genetic systems in the bacteria. This system enables some bacterial strains to cross the acidic pathways located in the stomach and thus stabilize the bacteria in the gastrointestinal tract and cause severe infections (1-3).

S. aureus bacteria is one of the most common pathogens causing disease and mortality. It can cause skin and soft tissue infections or even as an invasive, causative agent causes diseases such as pneumonia, endocarditis and septicemia. In fact, it is a symbiotic microbial agent in the body that is typically found as microbiome and in the nasal area without causing any disease (4,5). Carrying this bacterium in the nasal may be responsible for serious infections in hospitalized patients (6). In recent years, *S. aureus* resisting to methicillin (MRSA) is one of the main contributors to the development of hospital-acquired infections

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globally and reports suggest that approximately 05% of infections in ICUs are due to MRSA (7). The main source of bacterial presence is in therapeutic centers like the beds of patients and the skin of the staff, as well as infections of the skin (8,9). In addition to these, the bacterium causes food poisoning due to food contamination. In other cases, it also occurs in the burning of appliances or surfaces on which the food is cooked. This bacterium can produce poison at room temperature and cause poisoning (8-10).

Here are some details about the properties of the herbs that we are studying:

1- *Hypericum perforatum* is a durable herb of the Hypericaceae herbaceous, which also called tea grass, Hypercom, or thousand eyes, and in English, St. John's wort. The herb tea grass has a lot of therapeutic effects, the most important of which is anti-depressant (11,12). The plant is toxic and sometimes mistakenly called mountain tea. It is used to treat enuresis, fatigue, neuropathic pains, AIDS, genital infections, kidney stones, asthma, and tuberculosis. The effective ingredient of this herb is hypericin. It also contains vitamins E, A. Flavonoids in the extract of tea grass are structurally similar to synthetic amino oxidase inhibitors. The flavonoids are toloxatone and brofaromine. Also, the xanthones in the plant has the property of enzyme inhibition (13).

2- *Alcea (Althaea officinalis)* from the family of Malvancea. Althaea officinalis is beneficially effective in the treatment of diseases of liver and facial mastitis and its medicinal properties is due to its moderate nature. It is a strong laxative, expectorant, anti-cough, prevention of abdominal swelling, and anti-colic, and seed of this plant is also useful in the treatment of gastric ulcer. It is useful in the treatment of prostate and cervix inflammation. Its chemical compounds are glazed and mucilaginous. It contains starchy, fat, essential oils, anthocyanin, altheine, dioxybenzoic acid and cyanidine (14).

3- *Adiantum capillus-veneris* is herbaceous, warm and dry, clearing the cricks and kidney stones crushing. It is useful for treating colds, treating fever and whooping cough, blood purification, infection and bladder insufficiency, and spleen pain. Adiantum capillus-veneris herb is identified in the Iranian Gilaki dialect, the Sialk and Sialk wells, in Lori, the Persiuvosh and in the Kurdish dialect called Siyavashi. Adiantum capillus-veneris is moderate in terms of ancient medicine in Iran. In ancient medicine, Adiantum capillus-veneris herb was used to treat cough and cold symptoms and regional hair alopecia. The chemical compounds of its leaves contain mucilage, glucose, gallic acid, tartaric acid, tannin, essential oils and bitter substance called capillarine (15).

4- *Achilles millefolium* also called thousand leaves grass. This aromatic herb has essence, inulin and a little tannin, and is used in Europe as a medicine to stop fever. Its use is for the treatment of epilepsy, pneumonia, measles, rubella, chicken pox, tuberculosis, and chronic gastritis. Its fumigation and decoction are useful for treating catarrh, so it is very useful at the time of the cold. This herb contains protein, fat, carbohydrate, calcium, and thebaine. It is sudoriferous, analgesic, mucosal anxiety, anti-allergic, decongestant, sputum, peripheral vascular relaxant, hypertensive reducer, cerebral and arterial anti-thrombosis, varicose veins strengthener, wound and injuries healer, bleeding closure, monthly period agent, period regulator, monthly bleeding and pain reducer, deodorant healer, anti-dandruff, and skin moisturizer. Chemicals: volatile oils: kamazulen, camphor, sesqui terpene and lactone chilblain (16, 19).

5- *Artemisia absinthium* (Wormwood) is a plant used in traditional medicine for the treatment of intestinal parasites. Due to its Thujone, has a psychedelic effect. It is anti-parasite, monthly period agent, refrigerant and using for preventing stroke, various cancers, period disorders, nervous disorders, insomnia, arthritis, dyspepsia, jaundice, inflammation of the spleen, malaria, and hepatitis. Other names are: Great wormwood, Kharagoosh, Sinusitis, Roman wormwood, Absinthe, Affinition, Wormwood, Absinthetine. Its flower chemical composition contains essence consisting of 53% alpha, alcohols, and chamazulenes 3.6%, dihydrochamazulene, bisabolene, cadinene, phellandrene, sesquiterpene, lactones, such as arthabesin, also this herb contains flavonoids, phenolic acids such as vanillin acid, and comerica acid (17).

6- *Lavandula officinalis Syn.L*. herb which leaves and the green part of the plant have volatile essential oil, lavender is rich in flavors in its leaves and fuzzes, which is a refreshing, tonic, disinfectant and antiseptic. For weak nerves, epilepsy, spasms, malaria, insomnia, palpitations, facial eruption, whooping cough, toothache, colitis. Its other names are: peppermint benefit (Kheirodasht), Anus Olarvah, Lavand, Momsek, Kharami, and Lavand flower. Chemical composition: lavender essential oil has about 04% of linalyl acetate, also, there are compounds such as butyric acid, propionic acid and valeric acid, free linalyl and germabol (14, 16, 18).

7- *Ruta graveolens* is a durable herbaceous with branched and woody stems, 2-3 partial alternating leaves smooth and friable, the rue's species can be referred to as the mountains Ruta and wall rue. Ruta is carminative, anti-spasm, emetic, sedative, anti-inflammation, anti-microbial, and blood pressure reducer. Medicinal properties: Heart regulator, stimulating the secretion of the bile and repels the intestinal parasites, usefulness for joint pain.

Chemical compounds: The most important compounds of the airborne part include coumarins, furanocoumarins, furanquinolyne alkaloids, caprynic, caprylic, vitamin K and essential oils, the most important of which is methyl neonyl ketone, which is

used in the perfume industry and industrial flavors (14, 16, and 19).
8- *Pimpinella anisum* is a stimulant, aromatic, digestible, sputtering, warm, dry, tonic, and vasodilator with very aromatic seeds, which are also called Roman Badian and is very similar to fennel. Today, several medicinal properties have been identified for anisum including anti-bacterial and anti-fungal effects. It is also useful for headaches, dizziness, asthma, and sinusitis (15, 20).

2. Materials and Methods

In this study, the antimicrobial activity of aqueous and alcoholic extracts of Aphrodisiacs, Yarrow, Gotha, Goliath, Ansium, Perissauchan, Lavender and Ruta herbs on *E. coli* (ATCC 43895) and *S aureus* (ATCC25923) as standard bacteria were studied. Standard bacterial specimens of 0.5 Mac-Farmland were activated and inoculated on the Muller Hinton Agar medium and the cultured plates were incubated at 37° C for 48 hours. After observing growth, coloration and the gram-positive and -negative properties of cocci and coccus were assessed (4, 6, 21).

2.1. Determination of sensitivity to antibiotics and aqueous and alcoholic extracts of the plants

2.1.1. Methanolic extraction

Extracting by pure methanolic solvent (Merck Co., Germany) and using the method of maceration (alcoholic extraction) is carried out using standard methods. To extract, first 70 g of each dry plant were weighed and then methanol was added to it and were placed for 24 hours at the laboratory temperature. Next day, the extracts were strained by Watman-1 paper and then each plant extraction is left in water bath 100ºC to make a concentrated extract and resin gum. The resulting extracts were then put into an autoclave after purification to prevent contamination during work. After the autoclave was used with serial dilution, up to eight serial dilutions from each plant were made. We added sterile distilled water to the first tube of each plant extraction at a rate of 1g per ml. In subsequent tubes, 0.5 ml of distilled sterilized water was used in proportion to the dilution of the plant extract. Dilution values of 1/2, 1/4, and 1/8 were obtained (5, 22).

2.1.2. Aqueous extraction

At first, 70 g of each plant was weighed, then sterilized diluted water was added and placed on boiling heat for 15 minutes. Next, the extract was filtered with Whatman-1 filter paper and then obtained extract of each plant was placed in 100°C water bath so that the extract was concentrated and gum-like. The obtained extracts were put into an autoclave, after purification. After the autoclave using by serial dilution were applied to make eight dilutions from each plant. We added sterile distilled water to the first tube of each extracted plant at the rate of 1 g per ml. In the next tubes, 0.5 ml of distilled water was used and the herbal extracts were used in proportion to their dilutions. Dilution values of 1/2, 1/4, and 1/8 were obtained.

2.2. Determination of sensitivity was performed using Kirby-Bauer standard disk diffusion method

For testing the mentioned bacterial colonies (Staphylococcus and Escherichia bacteria), equivalent to 0.5 McFarland suspensions were prepared and then an amount of 20 µl of suspension was placed on plates and was diffused at the surface of Muller Hinton agar media. Then, discs containing 10 µl of alcoholic and aqueous extracts of the plants were placed at standard intervals. After 24 hours of incubation at 35° C, the diameter of the inhibition zone for each extract was measured according to the related table. Then, the results were evaluated for each sample and the antimicrobial properties of the extracts were recorded as susceptible and resistant according to the instructions (5, 7, 23, 24).

Table 1: Efficacy of methanolic and aqueous extracts at different dilutions

Sensitive population Percent						sensitive population percent					
Fifth concentration	Fourth concentration	Third concentration	Second concentration	First concentration	Methanolic extract	Fifth concentration	Fourth concentration	Third concentration	Second concentration	First concentration	Aqueous extract
85%	14%	42%	57%	72%	*Ruta graveolens*	72%	70%	72%	72%	100%	*Ruta graveolens*
42%	57%	71%	71%	82%	*Achillea millefolium*	58%	72%	72%	75%	83%	*Achillea millefolium*
57%	86%	84%	92%	95%	*Lavandula officinalis syn.l. anyustifolia*	57%	62%	65%	71%	85%	*Lavandula officinalis syn.l. anyustifolia*
28%	42%	42%	43%	72%	*Althaea officinalis*	15%	57%	78%	78%	100%	*Althaea officinalis*
57%	72%	72%	72%	81%	*Pimpinella anisum*	58%	55%	57%	71%	71%	*Pimpinella anisum*
42%	58%	72%	72%	72%	*Hypericum perforatum*	15%	57%	72%	72%	72%	*Hypericum perforatum*
43%	57%	72%	72%	72%	*Adiantum capillus veneris*	28%	39%	42%	65%	72%	*Adiantum capillus veneris*
57%	72%	86%	86%	86%	*Artemisia absinthium*	15%	43%	28%	57%	71%	*Artemisia absinthium*

3. Results

In this study, eight mentioned herb species which are listed in "Iranian Traditional Medicine" book were studied. Currently, no similar laboratory studies have been performed on these herbs and the case bacteria. To obtain the same results, the tests were repeated three times and the results were analyzed by SPSS software version 16 and after drawing the charts, the alcoholic and aqueous extract of *Artemisia absinthium* had the most effect on the *Escherichia coli*, and alcoholic and aqueous extract of yarrow *(Achillea millefolium)* had the most effect on *Staphylococcus aureus* (Table 1) that shows a lot of antibacterial effects from the aqueous and alcoholic extracts of *Artemisia absinthium* (Figures 1-3).

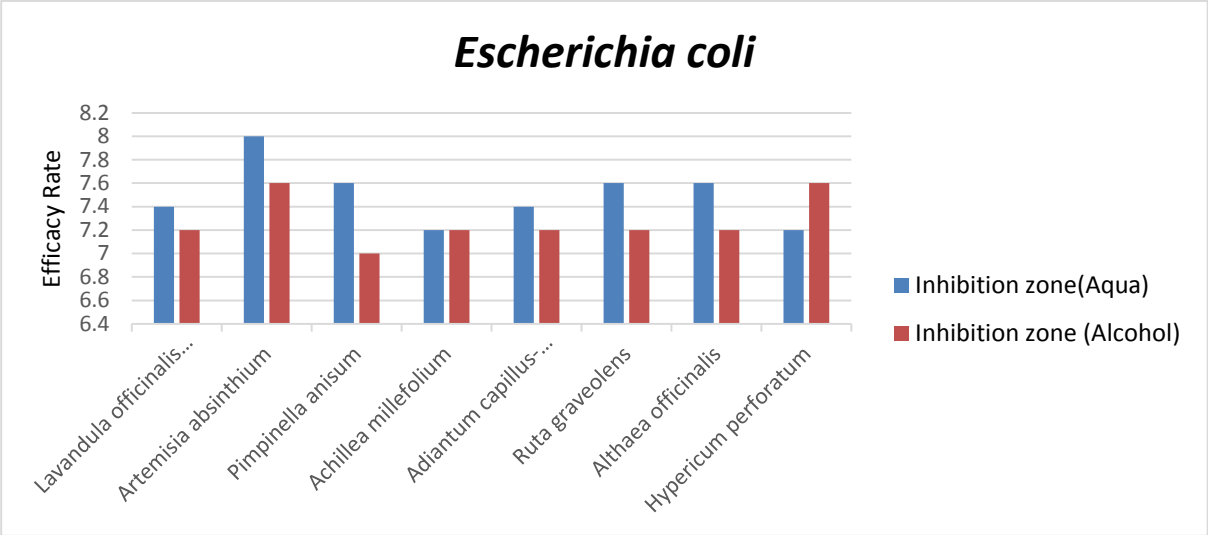

Figure 1. Efficacy rates of alcoholic and aqueous herbs extracts on *E. coli* bacteria

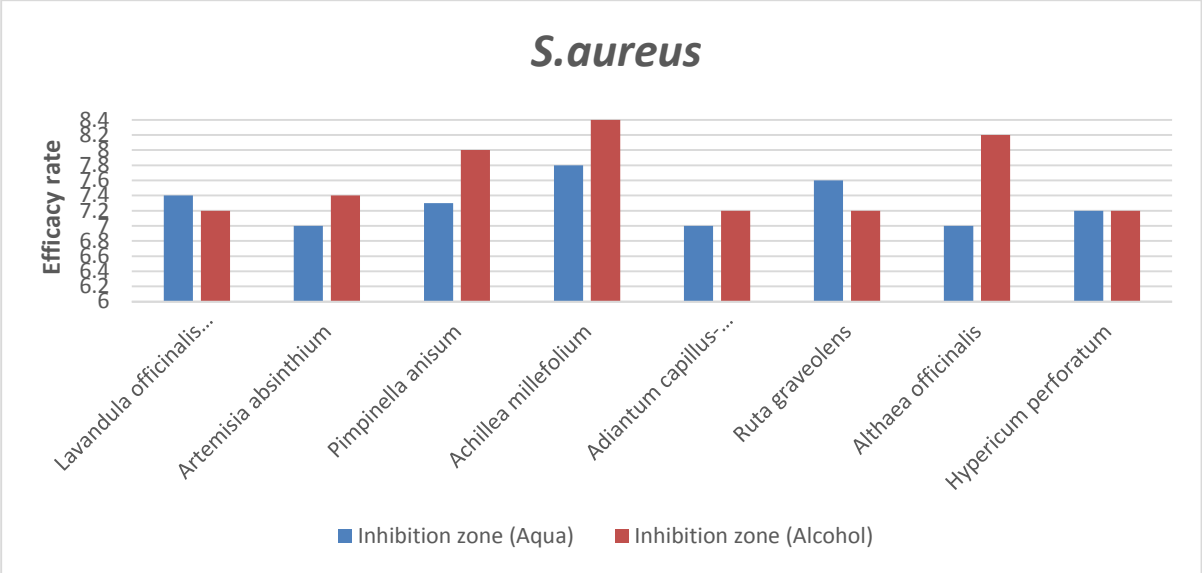

Figure 2. Efficacy rates of alcoholic and aqueous extracts on *S. aureus* bacteria

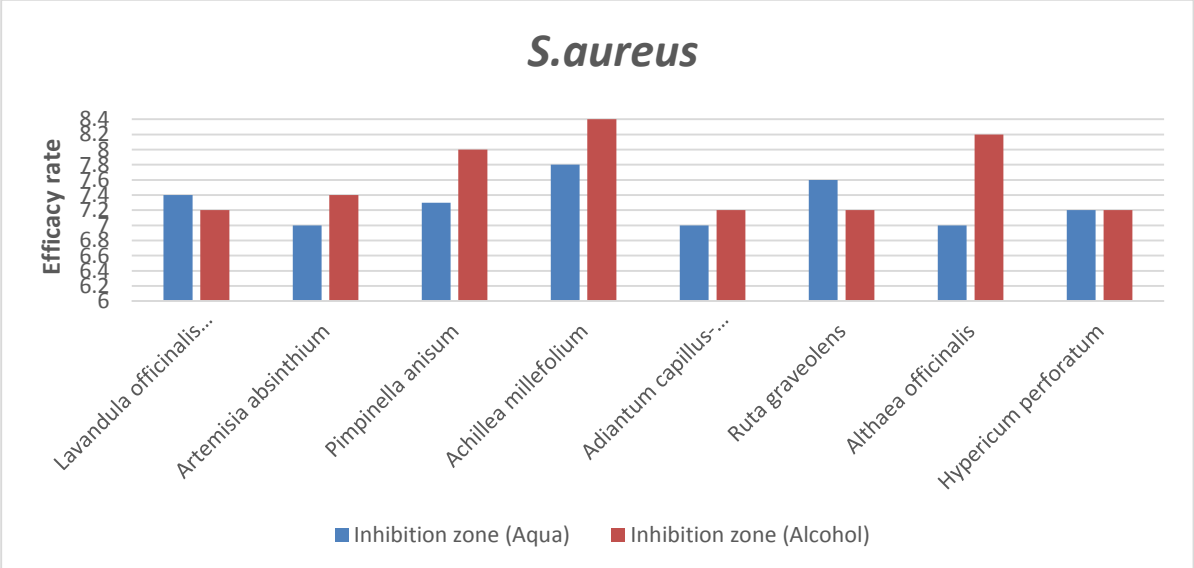

Figure 3. Comparison of antibacterial sensitivity percent of alcoholic and aqueous herb extracts

4. Discussion

According to Kohler CD et al research on the effects of Artemisia, Lavender, and Shirazi Vetiyah plants on bacteria *Pseudomonas aeruginosa*, Staphylococcus aureus and *Klebsiella pneumonia*, lavender essential oil had an inhibitory effect on the pathogenic bacteria studied (24). In the study on Anisone aqueous, methanolic, and acetone extracts' effect on 4 bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, and Klebsiella pneumoniae)*, it was shown that the aqueous and methanolic extracts of Anisone destroyed all bacteria and the aqueous extracts were more effective than methanolic extracts (4).

In another study, it was shown that Anisone does not have the ability to inhibit Gram-negative bacteria, but its alcoholic extract can inhibit the activity of the bacteria Mycobacterium and *Micrococcus smegmatis* (9). In another study, when the extract of this plant was used with thyme extract *(Thymus vulgaris)*, its inhibitory effect on the bacteria *Proteus vulgaris*, *Staphylococcus aureus*, and *Bacillus cereus* was greater.

Kosalec et al investigated the antifungal activity of Anisone essential oil on 7 yeast species and 4 species of dermatophytes, which showed that the Anisone extract could be effective to avoid the activity of fungus like *C. tropicalis, C. parapsilosis, Candida albicans, C. krusei and C. pseudotropicalis* (5, 24). In a study by Khan UA et al on separated isolates with multiple resistance, the Alkane ethanolic extract had an antimicrobial effect with a diameter of 11 mm for non-growth zone on *Acinetobacter baumannii* and it was 41 mm for *Escherichia coli* and 19 mm for *Staphylococcus aureus* (23).

The increasing medicine resistance in bacteria has led to more attention to be paid to find ways to prevent antibiotic resistance. For this reason, medicinal herbs have also been given special attention. The idea that plants have therapeutic properties arise from many years ago. Certain plants have long times been known as antimicrobial agents and are now also used (5, 24).

By observing the antimicrobial effects of the studied extracts in this research on the growth of *Escherichia coli and Staphylococcus aureus* strains, it is hoped that in the near future, by purification of the effective substances of the above-mentioned plants and performing further research, compounds with acceptable antimicrobial effects and less side effects will be achieved. In this field, it also appears to be necessary to perform experiments on animal models (in vivo) and also to assess the pharmacokinetic effects of plant extracts.

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