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Investigation of Antibacterial Effect of *Ferula macrocolea* Extract and Quantity Determination of Inhibitory Effect on 4 Standard Strains of Gram Positive and Gram Negative Bacteria

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Abstract

Objective: *Ficus exasperata* Vahl (Sandpaper tree) is extensively used in Nigeria to treat diseases, but a dearth of documentation about its toxicity exists. This information is crucial because pollutants can contaminate medicinal plants. This study determined the heavy metal and phytochemical content of methanolic leaf extract of *F. exasperata* obtained in Birnin Kebbi, Nigeria.

Material and Methods: The lethality of the plant was also assessed using 70 wild shrimps divided equally into seven groups. Group 1 (negative control), groups 2 and 3 (positive controls) were exposed to 500 and 1000 ppm of formaldehyde, respectively; and groups 4-7 were exposed to 1000, 2000, 4000, and 8000 ppm of extracts, respectively, for 96 hours.

Results: The phytochemistry revealed high levels of flavonoids and saponins and moderate levels of tannins and phenols. The heavy metal analysis revealed non-tolerable levels of cadmium, copper, and lead, while zinc was within the tolerable limit. The negative control recorded 10% mortality, 1000 and 2000 ppm (20% each), 4000 ppm (70%), and 8000 ppm (100%).

Conclusion: These results inferred safe doses of the plant's extract in low and medium concentrations but toxic and fatal at high doses over a period of time. Consumers are advised to seek an expert's guidance before using it.

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Introduction

Ferula which belongs to the genus Peucedaneae, the subfamily of Apioideae and family of Umbelliferae, is a perennial plant [1]. There are 30 species of Ferula in Iran, of which 15 species are known as native. The common Persian name of this plant is "coma" [2]. Some species of Ferula have long been used as traditional antispasmodics, sedatives, laxatives, disinfectants, expectorants, and in the treatment of colds and gastrointestinal diseases [3].

Numerous studies have been performed on the chemical composition and effects of different Ferula species. The results show that this genus contains important biochemical compounds including derivatives of Sesquiterpens [4].

The essential oil of a concentrated and complex liquid contains a variety of different compounds. These volatile compounds are synthesized by aromatic plants as secondary metabolites and act as the plant's defense system against bacteria, viruses, fungi, and insects [4].

According to the history of Iran in the field of medical treatment traditionally, with the use of medicinal plants and the growth of useful medicinal plants in our country, it is necessary to study the effects of different plant species. Due to the arbitrary and high use of antibiotics in the treatment of diseases and the development of bacterial resistance, the use of plants and their compounds, including essential oils and extracts, can be considered as an effective alternative to chemical drugs.

Escherichia coli is a gram-negative, which one of the most important members of the Enterobacteriaceae family and is a common cause of urinary tract infections (UTIs as well as gastroenteritis, especially in children and is the cause of 90% of urinary tract infections in young women [5]. This bacterium is the only member of this family that is considered both a pathogen and an opportunist [6]. This bacterium causes diarrhea, bleeding intestines and even side effects from hemolytic uremic syndrome (HUS) [7].

Staphylococcus aureus, which belongs to the micrococcaceae family, is a gram-positive, immobile, spore-free, facultative anaerobe bacterium and is one of the most well-known invasive bacteria that causes disease in humans and animals and is a major cause of nosocomial infection with a relatively high prevalence. This microorganism is involved in causing a wide range of diseases, including endocarditis, osteomyelitis, pneumonia, toxic shock syndrome, furuncle, etc. [8-10].

Staphylococcus epidermidis is the most important member of the coagulase-negative staphylococcus group and is the cause of 75% of infections in this group. These bacteria are gram-positive cocci that are often found in binary, chain, and cluster forms. This microorganism is found in air, food and water and is part of the natural microflora of human skin and is located in the nasal mucosa and upper respiratory tract. This bacterium is the third leading cause of nosocomial infections and one of the most common causes of bloodstream infections [11, 12].

The increasing prevalence of antibiotic resistance strains is one of the health problems that has led to a decrease in the number of effective and available antibiotics in the treatment of infections caused by bacteria. *E. coli*, *S. aureus*, *P. aeruginosa* are among the most important causative agents of nosocomial infections [13].

The aim of this study was to use the essential oil of *F. macrocolea* to investigate the antibacterial effects on four gram-positive and gram-negative bacteria. According to our research, no study has been done on the volatile compounds of this species. The growing prevalence of antibiotic resistance strains.

Materials and Methods

Extraction of Ferula essential oil

The reason for using these strains is their pathogenicity, which makes it necessary for treatment.

Preparation of microbial suspension

Microbial suspension was prepared from all strains in Muller-Hinton broth medium. Fresh cultures were prepared for each series of experiments. To do this, one loop of each microbe was inoculated in 5 cc solution of the above culture medium and incubated for 24 hours in 37 ° C. By adding 0.9% normal saline to the suspension and comparing its turbidity with 0.5 McFarland solution, a suspension with an approximate concentration of 1.5×108 microorganisms per milliliter was obtained, which was used for inoculation in Muller-Hinton agar medium.

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Evaluation of antibacterial properties of essential oil

To determine the qualitative and quantitative sensitivity, the prepared suspension was used.

The first method is the well drilling method

Using a drill bit, make a hole in a part of the culture medium and empty it completely. Then pour 100 microliters of essential oil directly into the well and place in a 37 ° C for 48 hours.

The second method is disk placement method:

Pour 25 microliters of the desired essential oil on the prepared antibiogram disks and place them on the culture medium. We also put it on the culture medium. By measuring the diameter of the auras formed around the plates, the results were evaluated and the results of antibiotics were compared with CLSI tables. To ensure each of the different concentrations of essential oils and antibiotics, these experiments were repeated three times for each bacterial strain.

Minimum inhibitory concentration (MIC) Quantitative method

The minimum concentration of growth inhibitor was determined by microdilution method. Based on the required concentrations, the essential oil was dissolved in BHI broth media containing 10% dimethyl sulfoxide (DMSO). Concentrations of 0.005, 0.01, 0.02, 0.04, 0.08, 0.16 were prepared. Bacterial suspension of 96 pollen wells was added to each well and two wells were used for each concentration of essential oil.

Results

The results of this study indicated essential oil of *F. macrocolea* had antibacterial effect on *S. epidermidis*, *B. subtilis*, *E. coli*, and *S. aureus* using broth microdilution and disc diffusion methods.

The results of antimicrobial effect of essential oil by quantitative and qualitative methods are shown in Tables 1 and 2.

Table 1. Results of determining the antimicrobial properties of essential oils by quantitative method

Bacteria	MBC	MIC
Staphylococcus epidermidis	The second well 50mL	The third well 25µL
Bacillus subtilis	Fourth well 12/5mL	The eighth well 6/28 µL
Escherichia coli	The third well 25mL	Fourth well 12/5 µL
Staphylococcus aureus	Fourth well 12/5mL	Fourth well 12/5 µL

Table 2. Results of qualitative antimicrobial properties of essential oils

Bacteria	Disk diffusion results
Staphylococcus epidermidis	11mm
Bacillus subtilis	23mm
Escherichia coli	16mm
Staphylococcus aureus	14mm

Results of micro-broth dilution

The results of determining the minimum inhibitory concentration of essential oil on four standard strains showed that it has the highest inhibitory effect on *B. subtilis*. Less antimicrobial effect was observed in *E. coli* and *S. aureus* strains than in *B. subtilis*. The minimum inhibitory concentration and the minimum bactericidal concentration of essential oil were similar for *S. aureus*.

S. epidermidis showed the least susceptibility to the antimicrobial effect of the essential oil.

Results of disc diffusion test

The results of disk diffusion and measurement of growth inhibition zoon showed that the essential oil has the greatest antimicrobial effect on *B. subtilis*.

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S. epidermidis showed less sensitivity to this essential oil than other bacteria.

Discussion

Today, due to the spread of antibiotic-resistant pathogens, control and treatment of these infections has become a serious problem. Therefore, finding compounds that have antimicrobial properties of antibiotics is very important [14].

It seems that in order to solve this problem and fight against antibiotic resistant pathogens, the use of plants in traditional medicine is a suitable strategy.

The results of various studies showed that the use of herbal medicines that have antimicrobial properties, significantly reduces the disadvantages of using chemical drugs, including increased treatment costs, side effects and increased antibiotic resistance [15].

Ferula species, especially *F. assa-foetida* and *F. gummosa*, have a long history in traditional medicine [2].

F. assa-foetida is a species that has antioxidant and antimicrobial properties. Recent studies have shown that this species also has antiviral properties against influenza virus (H1N1). Based on this, a study was performed on the above *F. assa-foetida* and the results showed sesquiterpene coumarins and essential oils have antiviral, cytotoxic, antibacterial and antifungal properties in this species [16].

Alireza Daneshkazemi et.al (2019) also investigated the antimicrobial properties of essential oil from *F. assa-foetida*. The results of this study showed that the two chemical compounds (Z-1-propenyl sec-butyl disulfide and (E) -1-propenyl sec-butyl disulfide) obtained from the seed extract of this plant have strong antibacterial properties [15].

Few studies have been performed on the biological and therapeutic activity of *F. macrocola*. Due to its anticonvulsant and antinociceptive properties, it has been proven that this plant is used in traditional medicine today [17].

In a study performed on ferrule extract, the effect of chemical compounds and their anti-apoptotic activity against hydatid cyst was investigated in vitro and in vivo [17].

Ferula hermonis is another species that was shown to have an antimicrobial effect of resin extract on gram-negative bacteria and the antimicrobial effect of its root oil extract on gram-positive bacteria [18].

In a similar study conducted by Zohreh Sattar and Mehrdad Iranshahi (2016), The antibacterial effect of this species was investigated and the results of this study showed that *F. hermonis* has a very strong antimicrobial effect against gram-positive bacteria, especially *S. aureus* and *Micrococcus luteus*, compared to gram-negative bacteria [19].

In the studies of Enis Fuat et al, The antimicrobial effect of another species called Ferula elaeochytris Korovin was evaluated. The aim of this study was to investigate the anti-Quorum sensing (QS) effect of this species. The results of this study showed that the extract of this species is a good candidate as an anti-(QS) compound against bacteria [14].

Because no study has been performed on the antibacterial effect of *F. macrocolea*, in our study, the antimicrobial effect of this species was investigated against several standard strains that are clinically relevant.

The results of the present study showed that the highest antimicrobial effect of this essential oil was on *B. subtilis* and the lowest effect was on *S. aureus*.

In our study, ferula extract had different inhibitory and lethal effects on all standard gram-positive and gram-negative strains in this experiment.

In the present study, the highest and lowest antibacterial effects of the extract according to Table 2, were on *B. subtilis* and *S. aureus*, respectively. Determination of minimum inhibitory concentration and minimum bacteriocidal concentration of the extract on standard strains confirmed the results of disk diffusion method.

In this study, it was shown that a very small concentration of extract would be effective in inhibiting and killing *B. subtilis* strain, while *S. aureus* strain would be inhibited in higher concentrations of this extract.

Conclusion

According to the results of the antimicrobial effect of Ferula extract, it seems that this plant has effective substances that in different concentrations are able to inhibit and kill various gram positive and negative bacteria. Examining the extracts of different parts of Ferula and determining its inhibitory and lethal concentrations on different bacteria can give a better understanding of the antibacterial properties of this plant.

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