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In vitro Evaluation of Antibacterial Activity of *Umbilicus intermedius Boiss, Cuminum cyminum* and *Zingiber officinale* Ethanolic Extracts

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ABSTRACT

Introduction: The use of medicinal plants for treatment of fungal and bacterial diseases has a long history. Today, because of the high resistance of microorganisms to antibiotics and chemical drugs side effects, the use of herbal medicines has increased. The aim of the present study was to evaluate the antibacterial activity of the crude ethanolic extract of Umbilicus intermedius Boiss, Cuminum cyminum and Zingiber officinale against some drug resistant bacteria.

Material and Methods: In this study the antimicrobial activity of Umbilicus intermedius Boiss, Cuminum cyminum (Cumin) and Zingiber officinale (ginger) has been evaluated against five multidrug resistant clinical bacterial isolates. The different concentrations ranged from 50 to 200 µg/ml of ethanolic extract of the three plants had been assayed separately against multidrug resistant Escherichia coli, Pseudomonas aeruginosa, Streptococcus agalactiae, and two isolates of Staphylococcus aureus. The antibacterial activity was determined by the disc diffusion method.

Results: Among the three evaluated herbs, only the cumin and ginger showed anti-bacterial activity. Among the extracts tested, ginger extract was the most effective. However, the ethanolic extract of Umbilicus intermedius Boiss showed no inhibitory effects on bacterial isolates at all concentration.

Conclusions: The findings of the present study indicate that ginger extract has good antibacterial effects. Conceivably, the use of herbal extracts such as ginger represents a new era for antimicrobial therapy.

Key words: Antibacterial activity, Disc diffusion, *Zingiber officinale, Umbilicus intermedius Boiss, Cuminum cyminum*

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INTRODUCTION

Today, the research and development of the new drug discovery from natural resources have become very important worldwide [1]. Nearly, 11 percent of the total 252 drugs in the World Health Organization's (WHO) crucial medicine list have an herbal source [2]. Natural products are the main source of new medicines, and their use as an alternative drug to cure various diseases has increased in the last few decades. The herbs and spices have fewer side effects than formulated drugs. Furthermore, they are cheaper, show more patient tolerance and easily accessible for lower social and economic populations [3-5]. The medicinal plants have been traditionally employed by people for various purposes in different parts of Iran [6]. The excessive use of antibiotics in infection treating has led to the emergence of resistant bacterial isolates, which is constantly increasing [7]. As a result, recent investigations are performed to find more effective natural anti-bacterial drugs with fewer side effects [8]. Three herbs that have been proven to have a medical use include *Umbilicus*

intermedius Boiss (Nafe Venus), *Cuminum cyminum* (Cumin) and *Zingiber officinale* (ginger).

So far, several studies have been conducted on anti-bacterial effects of ginger and cumin in the various countries, including Iran [3,8−10]. However, according to our data, unlike previous two herbs, there has not been much research on anti-bacterial effects of *U. intermedius Boiss.* Ginger has long been used in the medical field in the treatment of the different diseases including vomiting, diarrhea and epigastric pain. Additionally, side effects have not been observed in ginger consumption [11]. Cumin is an aromatic plant cultivated in India, China, Saudi Arabia and the countries along the Mediterranean Sea. It is used in toothpastes, mouthwashes and soaps preparations. It is also used as appetite stimulating and antispasmodic agent in medicine [9,12]. *Umbilicus intermedius Boiss* (Nafe Venus) is important medicinal plant that grows particularly in the west regions of Iran, such as Ilam city. It is traditionally used to treat various infectious diseases [13].

In the present study, we tested the hypothesis that crude ethanolic extract of *Umbilicus intermedius Boiss, Cuminum cyminum* and *Zingiber officinale* would show antibacterial properties against some drug resistant bacteria isolated from clinical samples, using disc diffusion method in laboratory assays.

MATERIALS AND METHODS

Collection of plant materials

The *U. intermedius Boiss* plant collected from the mountains of Ilam city, west of Iran. Cumin and ginger used in the present study were purchased from the local spice shops of Ahvaz and Yazd cities, Iran. All the plant materials were identified at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. The different plant parts were washed with water, air-dried, pulverized in a mill and stored in a sterile air-tight container until further investigation.

Plant extract preparation

Ethanolic extract of *Umbilicus intermedius Boiss*

Extraction was done by soaking of 10 g of plant powder in 250 ml of ethanol 80% for 72 h with shaking (150 rpm) at 25-30°C. The mixture was centrifuged to obtain the supernatant and then filtered twice through Whatman filter paper No. 1 (Whatman, Camlab, Norman way, UK). The alcohol was evaporated at 40°C using a rotary evaporator. The extract solution was carefully labeled and stored in sterile dark vials in a refrigerator at 4°C for further analysis.

Ethanolic extract of *Cuminum cyminum* and *Zingiber officinale*

Extraction was done by soaking of 100 g of each ginger and cumin powder in 500 ml of ethanol 96% for 72 h with shaking (150 rpm) at 25-30°C. The mixtures were centrifuged to obtain the supernatant and then filtered twice through Whatman filter paper No. 1. The alcohol was evaporated at 40°C using a rotary evaporator. The extract solutions was carefully labeled and stored in sterile dark vials in a refrigerator at 4°C for further analysis.

Test microorganisms

The panel of test bacteria for *in vitro* antibacterial screening is shown in Table 1. About 5 multidrug resistant (MDR) bacteria were isolated from patients of the teaching hospitals, affiliated to Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. All isolates were identified using standard microbiologic and biochemical tests including Gram-staining, colony characteristics and reaction on Triple Sugar Iron agar, Simmons' citrate agar and Christensen's urea [14]. The susceptibility patterns were obtained by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (Merck, Darmstadt, Germany) and results were interpreted based on the Clinical and Laboratory Standards Institute (CLSI) principles [15].

Table 1: Antibiotic resistance pattern of studied bacteria for *in vitro* antibacterial screening

Bacterial strain	Antibiotic resistance pattern
Escherichia coli	CRO, CTX, CAZ, FEP,GEN
Pseudomonas aeruginosa	CIP,CAZ, IPM, FEP, MEM
Streptococcus agalactiae	AM, FOX, CLI, P
Staphylococcus aureus 1	AM, FOX, ERY, P,CLI
Staphylococcus aureus 2	AM, FOX, CIP, P

AM: Ampicillin; CLI: Clindamycin; ERY: Erythromycin CRO: Ceftriaxone; CTX: Cefotaxime; FOX: Cefoxitin; CAZ: Ceftazidime; FEP: Cefepime; CIP: Ciprofloxacin; GEN: Gentamicin; IPM: Imipenem; MEM: Meropenem; P: Penicillin

Antibacterial assay of plant extracts

The antibacterial activity of plant extracts was performed using the disc diffusion method. The test organisms were sub cultured in 5% sheep blood agar plate (BAP) for 24 h at 37°C. The colonies were inoculated in normal saline solution and the turbidity was adjusted to equal the 0.5 McFarland standard. A total of 0.1 ml of bacterial suspension was lawn cultured on each plate, containing Muller-Hinton Agar, by sterile cotton swab. Sterile paper discs (6 mm diameter) were impregnated with 10 µl of three different concentrations (50,100 and 200 µg/ml) of the extracts and placed on the inoculated plates. The plates were incubated at 37°C for 24 h. Representative antibiotics of the test isolates based on susceptibility patterns were used as positive controls (Table 1). Antibacterial activity was recorded by measuring the diameters of inhibition zone (DIZ) in mm against the test bacteria. *E. coli ATCC 25922* was used for quality control.

RESULTS

The disc diffusion method for antimicrobial susceptibility testing was performed to determine the

antibacterial activities of the 3 medicinal plants against MDR bacteria, including two Gram negative strains (*E. coli* and *P. aeruginosa*) and three Gram positive strains (*S. agalactiae, S. aureus 1* and *S. aureus 2*). The results are shown in Table 2. Of the 3 medicinal plants, the ethanolic extracts of *C. cyminum* and *Z. officinale* showed inhibitory effects against all of the test bacteria except *E. coli*. The plant extracts demonstrated inhibition zones ranging from 6 mm to 24 mm diameter, with the most significant results shown by *Z. officinale.* The ethanolic extract prepared from the *C. cyminum* and *Z. officinale* exhibited greatest inhibition zones (12-24 mm) against the clinical strain of *S. aureus 2*, but failed to inhibit the growth of Gram negative E. coli tested. On the other hand, *U. intermedius Boiss* produced no inhibition zones of growth against all clinical bacteria tested. Among the Gram negative strains, *P. aeruginosa* showed the highest sensitivity to cumin and ginger extracts. Furthermore, none of the ethanolic extracts were able to inhibit the multidrug resistant *E. coli*.

Table 2: Diameter of inhibition zones (mm) of Ethanolic extracts from 3 medicinal plants against clinical isolates of MDR bacteria

Plant extracts (concentration mg/ml)	*E. coli*	*P. aeruginosa*	*S. agalactiae*	*S. aureus 1*	*S. aureus 2*
Z. officinale (50)	-	-	8	10	14
Z. officinale (100)	-	6	10	12	20
Z. officinale (200)	-	12	14	14	24
C. cyminum (50)	-	-	-	-	12
C. cyminum (100)	-	8	12	10	15
C. cyminum (200)	-	12	18	14	19
U. intermedius Boiss (50)	-	-	-	-	-
U. intermedius Boiss (100)	-	-	-	-	-
U. intermedius Boiss (200)	-	-	-	-	-

DISCUSSION

Since the rise of antibiotics in the late 40's of the last century, the use of plant derivatives as antimicrobial agents has been scarce, mainly because of the strong pressure from the pharmaceutical industry in favor of synthetic and semi-synthetic products [16]. However, the traditional use of medicinal plants for the treatment of bacterial infections has made them an important source of antimicrobial compounds [17]. In the discussion that follows, we interpret the results with respect to our main hypotheses. In the present study, we hypothesized that the ethanolic extracts of *U. intermedius Boiss, C. cyminum* and *Z. officinale* would be effective against some multidrug resistant clinical bacteria by disc diffusion method.

The results revealed that the *C. cyminum* and *Z. officinale* have antibacterial activity with variable degree against tested bacterial strains. The data showed in Table 2 indicates that *Z. officinale* ethanolic extract exhibited highest antibacterial activity against all tested MDR bacteria except *E. coli*. The antimicrobial activity shown by ginger extracts in this study agrees with the findings of previous studies [18,19]. The antibacterial effects of ginger against *P. aeruginosa* and *S. aureus* obtained in our study corroborate with the report of Gao and Zhang [20], which explains that organic extracts of dried ginger and processed ginger had an inhibitory effect on both Gram positive and Gram negative bacteria. Despite the fact that the anti *E. coli* properties of ginger have been shown in previous studies [8,18,21] , our experimental data did not show any inhibitory effects against *E. coli,* which is consistent with the research by Onyeagba et al. in this regard [22]. Ginger (*Zingiber officinale*), is a popular spice used globally especially in most of the Asian countries. Chemical analysis of ginger shows that it contains over 400 different compounds. The major constituents in ginger rhizomes are carbohydrates (50-70%), lipids (3%-8%), terpenes, and phenolic compounds include gingerol [23]. Its gingerol-related conponents have been reported to possess antimicrobial and antifungal properties [24]. Besides these, amino acids, raw fiber, ash, protein, phytosterols, vitamins (e.g., nicotinic acid and vitamin A), and minerals are also present [23]. Ginger has been reported as a pain relief for arthritis, muscle soreness, chest pain, low back pain, stomach pain, and menstrual pain. It can be used for treating upper respiratory tract infections, cough, and bronchitis. As an anti-inflammatory agent, it is recommended for joint problems [25].

The results given in Table 2 revealed that cumin ethanolic extract had lower antibacterial effects against *S. agalactiae* and *S. aureus* in comparison to the ginger extract, while its antibacterial effect on *P. aeruginosa* is higher than ginger. Furthermore, like the ginger and Nafe Venus, the cumin ethanolic extract also showed no antibacterial effect on multidrug resistant *E. coli* by disc diffusion method that agrees with the findings of Mostafa et al. [26]. Cumin and value added products from cumin are used in food flavoring and perfumery and can be a good source of nutraceuticals with many biological activities. Cumin contains volatile oil (3-4%), cuminaldehyde, the major active principle, which is present to an extent of 45-50%. Alcohol and water extract of cumin are reported to possess many nutraceutical properties like antiallergic, antioxidant, anti-platelet aggregation, and hypoglycemic [27].

In our study, *Umbilicus intermedius Boiss* doesn't exhibit any antimicrobial properties against all studied bacteria, however a previous study by Roozegar et al. confirmed that *U. intermedius Boiss* possess the antimicrobial action in different concentrations against species of gram negative and gram positive bacteria [13].

Numerous studies have been conducted on the antibacterial potential of medicinal plants and spices, which showed varied results. There are wide dissimilarities and contradictions between the results even against the same bacterial species, indicating that the efficacy

of plant products is greatly affected by many reasons such as the method of extraction, antibacterial assay conditions, genetic variations among bacterial strains and their sources [28,29]. The findings of current study have afforded the justification for therapeutic potential of spices. Further and additional detailed investigations, *in vivo*, are recommended to define the efficacy of these plants in the treatment of severe bacterial infections.

CONCLUSION

The study suggested that cumin and ginger ethanolic extracts were effective against the growth of tested bacteria and could be a potential source of new antibacterial agents, but *U. intermedius Boiss* had no effect on bacterial growth. In this study, we expected the *U. intermedius Boiss* extract to be effective on bacteria, but it did not come to any conclusion. Therefore, it is suggested that further studies be carried out to determine the antimicrobial ability of this plant.

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CONFLICT OF INTEREST

Authors declare there is no conflict.

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