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Effect of ethanolic extract of *Ecballium elaterium* against *Staphylococcus aureus* and *Candida albicans*

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ABSTRACT

Objective: To evaluate the antimicrobial activity of ethanolic extract of *Ecballium elaterium* (*E. elaterium*) fruits alone against *Staphylococcus aureus* (*S. aureus*) strains and *Candida albicans* (*C. albicans*) strains, or in combination with penicillin against *Staphylococcus areus* strains. **Methods:** Evaluation of the antimicrobial activity or synergy interaction was carried out using microdilution method. **Results:** The results showed that ethanolic extract of *E. elaterium* fruits has antimicrobial activity against methicillin resistant *S. aureus* (MRSA), methicillin sensitive *S. aureus* (MSSA) and *C. albicans*. This extract showed a significant decrease in minimum inhibitory concentrations (MIC) of penicillin against both MRSA and MSSA strains. Fractional inhibitory concentration index (FIC) between penicillin and ethanolic extract of *E. elaterium* fruits against these test strains was less than 0.5. **Conclusions:** This study suggests that ethanolic extract of *E. elaterium* fruits has antimicrobial activity against *S. aureus* and *C. albicans* and there is a possibility of concurrent use of penicillin and *E. elaterium* extract in combination in the treatment of infections caused by MRSA and MSSA strains. A wider study is needed to identify the effective components, the mode of action and the possible toxic effect *in vivo* of these ingredients.

1. Introduction

Infectious disease still represent an important cause of morbidity and mortality among humans, especially in developing countries. In recent years one of the more alarming trends in clinical microbiology has been the increasing incidence of resistance to antimicrobial agents among pathogens causing nosocomial as well as community-acquired infections. Among the more important emerging resistance problem is methicillin resistance in *Staphylococci*, which has gained much attention in the last decades, because it has become a major hospital-acquired pathogen[1]. Methicillin resistant *Staphylococcus aureus* (*S. aureus*) (MRSA) infections are very difficult to cure because these strains are resistant against almost all clinically available antibiotics. For most MRSA strains, glycopeptid-type drugs are the only effective antimicrobial agents. This reason and other factors such as high cost of production and toxicity of synthetic compounds have prompted scientists to look for new effective antimicrobials which are of great importance in the clinical health.

Medicinal plants have been important sources of products in treating common infections and overcoming the problems of resistance and side effects of the currently available antimicrobial agents in the developing countries. One therapeutic strategy employed to overcome these resistance is the use of combination of drugs, such as β-lactamase inhibitors together with β-lactams[2]. Concurrent administration of two or more drugs is often essential and sometimes mandatory in order to achieve the desired therapeutic goal or to treat co-existing diseases.

Plants are known to produce variety of phytochemicals and generally good for combination therapy which act as multidrug resistance modifiers[2]. These phytochemicals are not effective antimicrobial agents by themselves, but they can reverse the resistance by blocking the efflux pumps or other mechanisms. The efflux pump inhibitors from natural sources can be co-administered with the antibiotic to decrease the degree of resistance of the bacteria to the drugs, reverse the acquired resistance or reduce the emergence of resistant pathogen[3].

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Ecballium elaterium (*E. elaterium*), the squirting cucumber or spitting cucumber, is from the Cucurbitaceae family. It is a decumbent, perennial herb restricted to the Mediterranean Basin and cultivated in central Europe and England. This plant has hairy vine, palmately lobed, bristly leaves. The fruit is ovoid, fleshy, approximately 4 cm in length. The unripe fruit is of a pale green color, and covered with numerous, uniseriate glandular hairs, which eject dark seeds and juice after maturity in response to light pressure. It is common throughout the Mediterranean area as a medicinal plant[4-7]. *E. elaterium* is of interest today because its fruits extracts are still used in Mediterranean region in different medicinal system[5,8]. The diluted aqueous extract of *E. elaterium* fruits is a traditional anti-inflammatory and analgesic for chronic sinusitis. It also possesses other uses especially the treatment of fever, cancer, liver disorders, jaundice, constipation, hypertension, dropsy, rheumatic diseases, and fungicidal[6,9-11].

Cucurbitacin seems to be responsible for the major pharmacological and biological effects of *E. elaterium* plant. For example, due to its strong bitter taste, cucurbitacin acts as purgative agent by stimulating gastric secretion. Also it has been found to decrease the damage in the chronic hepatitis and is responsible for the antimicrobial, antifungal. It was found that the anti-inflammatory activity of cucurbitacin B isolated from the juice of *E. elaterium* may be due to a modification in leukotriene B4 production[6]. Cucurbitacins inhibit the proliferation of cancer cells through various mechanisms[12].

All parts of the squirting cucumber are toxic, particularly the ovoid green fruits. Several toxicity and allergic reactions have been described if used undiluted[4,7,13-16]. The mechanism of toxicity (*i.e.*, direct toxic effect, hypersensitivity response) is also not well defined, but the clinical effects often do not respond to antihistamines or epinephrine. Progression of the inflammation can cause conjunctival irritation, corneal edema and erosions, sore throat, dysphagia, drooling, dyspnea, or respiratory distress secondary to upper airway edema. Obstruction of the upper airway is a potentially fatal complication of the nasal installation of undiluted juice from the squirting cucumber. In general cucurbitacins and the extracts containing them are considered to be toxic, with degree of toxicity depending on the plant material, type of extract, and the substitution partner of the compound. For example, exposure to the juice of anti-inflammatory medicinal plant *E. elaterium*, especially in undiluted form, often lead supposedly inflammatory irritation of the mucous membranes. These toxic effects seem to correspond to the juice's major active compound, cucurbitacin B[6]. Using plants for medicinal purposes is an important part of the culture and the tradition in Palestine. Therefore, this *in vitro* study was aimed at screening of ethanolic extract of *E. elaterium* fruits for antimicrobial activity and evaluating its potential use in treating infections caused by methicillin resistant MRSA, methicillin sensitive *S. aureus* (MSSA) and *Candida albicans* (*C. albicans*).

2. Materials and methods

2.1. Plant extraction

The ripe fruits of *E. elaterium* were collected from An-Najah N. University campus, Nablus, Palestine, during late September of 2010. The fruits were homogenized, and then dried in incubator at 37 ℃. Exposure to light was avoided to prevent the loss of effective ingredients. Approximately 30-40 g of dried material were mixed thoroughly with magnetic stirrer in 200 mL of 80% ethanol at room temperature and the mixture left for 24 h. The insoluble materials were removed by centrifugation at 10 000 rpm for 10 min at 4 ℃. Then, the extract was evaporated to dryness at 37 ℃. The extract was weighed and dissolved in sterile distilled water at a concentration of 200 mg/mL and stored at 4 ℃ for assay.

2.2. Antimicrobial agents

In this study two antimicrobial agents were used, one is Penicillin G (Birzeit-Palestine Pharamaceutical Co.) which is antibacterial agent and used against *S. aureus*, while the other is Bifonazole (Pharmacare PLC, Palestine) which is antifungal agent. Penicillin G was diluted to a final concentration 200 U/mL, while Bifonazole was diluted to a final concentration 100 μg/mL.

2.3. Staphylococcus aureus strains

Seven clinical of *S. aureus* isolates, three MSSA strains and four MRSA strains were used to study the effect of ethanolic extract of *E. elaterium* fruits. These isolates were identified as *S. aureus* according to colonial and microscopic morphology, growth on mannitol salt agar, 5% blood sheep agar, positive catalase, and coagulase production. Methicillin resistance was carried out using the disk diffusion method as described by Bauer *et al*[17]. Methicillin resistance was also confirmed by PCR technique using primers described by Vannuffel *et al*[18]. Both disk diffusion method and PCR technique were done in the Microbiology Laboratories of An-Najah National Univesity, Palestine. Methicillin (5 μg) disks (Oxoid) were used and inhibition zones were determined in accordance with procedures described by Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards, NCCLS)[19]. A reference strain (*S. aureus* ATCC 25923) was also included.

2.4. Candida albicans strains

Three clinical strains of *C. albicans* were used in this study. *C. albicans* were differentiated from other *Candida*

and *Cryptococcus* species by its ability to grow on the Levine formula of EMB agar and to produce germ tubes within 3 h, and pseudohyphae and budding cells at 18–24 h when incubated at 35 ℃ in 5%–10% CO_2. The addition of tetracycline to the Levine formulation aids in the selection of *C. albicans* from clinical sources that are contaminated with bacteria. A reference strain (*C. albicans* ATCC 10231) was also included.

2.5. Microdilution method

Minimum inhibitory concentration (MIC) of penicillin alone, ethanolic extract of *E. elaterium* fruits alone and in combination were determined by the microdilution method as described by National Committee for Clinical Laboratory Standards (NCCLS)[20]. Penicillin and *E. elaterium* ethanolic extract were serially diluted in Mueller Hinton broth. In case of combination, 1/8–1/64 (0.024 mg/mL) of *E. elaterium* ethanolic extract MIC was added to the serially diluted penicillin. *S. aureus* inoculum size of 1×10^5 CFU/mL was added to each well. Controls with broth only, broth with *E. elaterium* ethanolic extract, and broth with bacteria were included in the experiments. Each test strain of bacteria was run in duplicate. The test plates were incubated at 37 ℃ for 18 h. The MIC was taken as the minimum concentration of the dilutions that inhibited the growth of the test microorganism. For evaluation of interaction between antibiotic and plant extract, fractional inhibitory concentration (FIC) and FIC index were calculated based on the following formulas: $FIC_A = MIC_A$ in combination/MIC_A alone, $FIC_P = MIC_P$ in combination/MIC_P alone, and the FIC index = $FIC_A + FIC_P$, where FIC_A, FIC_P, MIC_A and MIC_P are the FICs and MICs for antibiotic A and plant extract P, respectively. Synergism was defined as an FIC index less than 0.5, while antagonism was defined as an FIC index greater than 2[21].

MIC of Bifonazole alone and ethanolic extract of *E. elaterium* fruits alone were determined by the microdilution method as described by NCCLS[22]. Antifungal agent and *E. elaterium* ethanolic extract were serially diluted in RPMI 1640 medium (Sigma) buffered to pH 7.0 with 0.165 M morpholinopropansulfonic acid (MOPS) buffer (Sigma). The yeast inoculum was adjusted to a concentration 1×10^5 CFU/mL and was added to each well. Controls with medium only, medium with *E. elaterium* ethanolic extract and medium with yeast were included in the experiments. Each test strain of *C. albicans* was run in duplicate. The test plates were incubated at 35 ℃ for 24 h. The MIC was taken as the minimum concentration of the dilutions that inhibited the growth of the test microorganism.

Table 1
FIC index and MIC of penicillin alone, ethanolic extract of *E. elaterium* fruits alone and in combination against 7 clinical *S. aureus* isolates and a reference strain *S. aureus* ATCC 25923 using microdilution method.

S. aureus strain		MIC			
		Penicillin (U/mL)	*E. elaterium* (mg/mL)	Penicillin in combination with *E. elaterium* (U/mL)	FIC index
S. aureus ATCC 25923	MSSA	0.391	0.195	$<1.9\times10^{-4}$	<0.123
Strain 1	MSSA	0.195	0.195	$<1.9\times10^{-4}$	<0.124
Strain 2	MSSA	6.25	0.195	$<1.5\times10^{-3}$	<0.123
Strain 3	MSSA	50	0.780	0.391	0.109
Strain 4	MRSA	100	0.195	<0.024	<0.123
Strain 5	MRSA	>100	0.391	0.195	<0.063
Strain 6	MRSA	50	1.563	3.125	0.078
Strain 7	MRSA	>100	0.391	<0.012	<0.061

3. Results

Our results showed that MRSA, MSSA and *C. albicans* are susceptible organisms to ethanolic extract of *E. elaterium* fruits. The MIC values of ethanolic extract of *E. elaterium* fruits alone against *S. aureus* were in the range of 0.195–1.563 mg/mL including *S. aureus* ATCC 25923 as a reference strain. The MIC values of penicillin alone against *S. aureus* were in the range of 0.195 to more than 100 U/mL. In case of combination between penicillin and *E. elaterium* ethanolic extract, 1/8–1/64 (0.024 mg/mL) of *E. elaterium* ethanolic extract MIC reversed the high level resistance of MRSA to penicillin. Also it induced a supersusceptibility to penicillin in MSSA which does not express mecA. This was proved by a significant reduction in MICs against both MRSA and MSSA (Table 1). FIC between penicillin and *E. elaterium* ethanolic extract against these test strains were less than 0.5. FIC indices were presented in Table 1. The MIC values of *E. elaterium* ethanolic fruits extract against *C. albicans* strains were ranging from 0.048 8 to 6.250 0 mg/mL while for reference strain *C. albicans* ATCC 10231, the MIC was 0.097 7 mg/mL (Table 2).

Table 2
MIC of Bifonazole alone and ethanolic extract of *E. elaterium* fruits alone against 3 clinical isolates of *C. albicans* and a reference strain *C. albicans* ATCC 10231 using microdilution method.

C. albicans strain	MIC	
	Bifonazole (μg/mL)	*E. elaterium* (mg/mL)
C. albicans ATCC 10231	>50	0.097 7
Strain 1	>50	0.048 8
Strain 2	>50	0.048 8
Strain 3	>50	6.250 0

4. Discussion

Plants remain one of the main sources of natural products for new therapies particularly in poor countries, because most of them are cost less, affect a wide range of antibiotic resistant microorganisms, and another reason is there is an erroneous impression that herbal medicines have fewer adverse effects[23]. Organ toxicity has been observed associated with various herbal preparations involving the liver, kidneys, and the heart. Some herbs may have carcinogenic properties[24]. A major criticism associated with the use of herbal medicines is the absence of scientific evaluation of their safety profiles since many of them have turned out to be toxic[25].

No articles on antimicrobial activity of *E. elaterium* was published in PubMed. In this study the antibacterial activity of ethanolic extract of *E. elaterium* fruits was assessed *in vitro* when used alone against *S. aureus* or *C. albicans* or in combination with penicillin against *S. aureus*. The activity of the antibiotic against *S. aureus* was increased by the presence of sub-inhibitory concentration. Most clinical strains of *S. aureus* are resistant to penicillin and a high percent of these strains are methicillin resistant. In order to assess the effect of combinations of ethanolic extract and antibiotic, the MIC values for antibiotic were determined to be as a reference point for defining the interactions[26]. Synergistic effects resulting from the combination of antibiotics with various plant extracts have been studied earlier against MRSA or MSSA[27-41]. From this study, ethanolic extract of *E. elaterium* fruits is very efficient in treating infectious diseases caused by MRSA and may also helpful for treating diseases caused by *C. albicans*. However, to explain the mode of action, the active phytocompounds of these plants used against multidrug-resistant bacteria and their toxicity have to be determined by additional studies. It was suggested that the use of some agents that can modify the bacterial cell to produce a new phenotype that is susceptible to the antibiotic could be an alternative approach to the treatment of infectious disease[42]. Our results were consistent with the recent report by Oskay *et al*[1], which showed that *E. elaterium* ethanolic extract has antibiotic activity against multi-drug resistant bacteria using well diffusion method. Also our results were inconsistent with a recent report by Dogruoz *et al*[43], which showed that *E. elaterium* aqueous extract didn't show antibacterial activity using agar well diffusion against different species of bacteria. This may be due to using very low concentration (1 μg/mL) and aqueous extract.

In conclusion, the results presented in this report are encouraging. A wider study is needed to identify the effective components, the mode of action and the possible toxic effect *in vivo* of these ingredients. Also this study probably suggests the possibility of concurrent use of penicillin and ethanolic extract of *E. elaterium* fruits in combination in treating infections caused by *S. aureus* strains. The maximum benefit can be achieved when the pharmacokinetics of natural product and the antibiotic combination match. The optimal ratio and dosing regimens should be explored for higher efficacy and decreased toxicological profiles

Conflict of interest statement

We declare that we have no conflict of interest.

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