



Antimicrobial activity of plant extract Ankaferd Blood Stopper®

Nuriye Tasdelen Fisgin^{a,*}, Yeliz Tanriverdi Cayci^b, Ahmet Yilmaz Coban^b, Duzgun Ozatli^c, Esra Tanyel^a, Belma Durupinar^b, Necla Tulek^a

^a Ondokuz Mayıs University, Medical School, Department of Clinical Microbiology and Infectious Disease, 55139, Samsun, Turkey

^b Ondokuz Mayıs University Faculty of Medicine, Department of Microbiology and Clinical Microbiology, Samsun, Turkey

^c Ondokuz Mayıs University Faculty of Medicine, Department of Hematology, Samsun, Turkey

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ABSTRACT

The in vitro antimicrobial activity of Ankaferd Blood Stopper® (ABS) was assessed on 102 clinical isolates from both Gram negative and Gram positive bacteria and four standard strains, including MRSA ATCC 43300, MSSA ATCC 25923, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 35218. ABS was significantly active against all bacteria investigated.

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1. Introduction

Ankaferd Blood Stopper® (ABS) is a unique folkloric medicinal plant extract. The basic mechanism of action of ABS is through the formation of encapsulated protein network providing focal points for vital erythrocytes to aggregate on. The ABS induced protein network formation involves blood cells, particularly erythrocytes, without affecting the physiological individual coagulation systems. ABS is a standardized extract from the following plants: *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum* and *Urtica dioica* in a weight ratio of 6:8:7:7:5, respectively [1,2]. The antibacterial activity of these plants has been investigated previously [3–9]. Thus, *Thymus vulgaris* has shown bacteriostatic activity for Gram positive and Gram negative bacteria [3–6], as well as *Glycyrrhiza glabra*, *Vitis vinifera* and *Alpinia officinarum* have been shown to be antibacterial [7,8], with *Urtica dioica* being endowed with noticeable antibacterial activity against *Streptococcus pyogenes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* [8]. Each of these plants has some effect on cellular proliferation, blood cells, the endothelium and vascular dynamics [10–13]. Since the antimicrobial activity of ABS is currently unknown, we aimed to

evaluate the activity of ABS against a series of bacteria of clinical relevance that includes *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter* spp., *Stenotrophomonas maltophilia*, methicillin resistant *Staphylococcus aureus* (MRSA), methicillin susceptible *S. aureus* (MSSA), methicillin resistant coagulase negative *Staphylococcus* (MRKNS), vancomycin susceptible *Enterococcus* and vancomycin resistant *Enterococcus* (VRE).

2. Materials and methods

2.1. Ankaferd Blood Stopper®

ABS was obtained from Trend Teknoloji Ilac AS, Istanbul, Turkey as a solution for direct application, spraying or incorporation in dressing material to injured skin or mucosa. Ankaferd solution is a registered product of a combination of plant extracts. Relative ratio of plants extraction is shown below.

	Amount (g/100 ml)
<i>Urtica dioica</i>	6.0
<i>Vitis vinifera</i>	8.0
<i>Glycyrrhiza glabra</i>	7.0
<i>Alpinia officinarum</i>	7.0
<i>Thymus vulgaris</i>	5.0

* Corresponding author. Tel.: +90 362 3121919/3175; fax: +90 362 4576041.
E-mail address: nuriyef@omu.edu.tr (N. Tasdelen Fisgin).

Table 1
Distribution bacterial strains according to clinical specimens

	Blood culture	Tracheal aspiration culture	Wound culture	CSF culture	Urine culture	Total
<i>A. baumannii</i>	1	4	2	1	2	10
<i>E. coli</i>	–	–	2	–	7	9
<i>K. pneumonia</i>	–	1	1	–	8	10
<i>Enterobacter</i> spp.	2	1	1	1	5	10
<i>S. maltophilia</i>	2	7	1	–	–	10
<i>P. aeruginosa</i>	1	5	1	–	2	9
MRSA	1	5	2	1	–	9
MSSA	2	–	4	–	1	7
MRKNS	5	–	1	2	1	9
<i>Enterococcus</i> spp.	2	–	2	–	5	9
Total	16	23	17	5	31	92

MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-sensitive *Staphylococcus aureus*, MRKNS: Methicillin-resistant coagulase negative staphylococci, CSF: Cerebro-spinal fluid.

The amounts given above are sample figures only and the invention is not limited to these amounts. Compositions of the invention can be prepared with different amounts, proportions and concentrations of drugs according to intended application and case [2].

2.2. Bacterial strains

One hundred two clinical isolates were tested, including 10 *A. baumannii*, 9 extended spectrum beta lactamase (ESBL) positive *E. coli*, 10 ESBL positive *K. pneumonia*, 9 *P. aeruginosa*, 10 *Enterobacter* spp. (6 *Enterobacter cloacae* and 4 *Enterobacter aerogenes*), 10 *S. maltophilia*, 9 MRSA, 7 MSSA, 9 MRKNS, 9 vancomycin susceptible *Enterococcus* (6 *Enterococcus faecalis* and 4 *Enterococcus faecium*) and 10 VRE (Table 1). All isolates were isolated from various clinical specimens at Ondokuz Mayıs University Medical School, Microbiology Laboratory in 2007. We also tested MRSA ATCC 43300, MSSA ATCC 25923, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 35218 standard strains.

2.3. Preparation of inoculums

A modified methodology for investigation of antibacterial activity of ABS was used. We prepared disc containing 25 µl ABS and then it was tested by the disc diffusion method [14]. However 25 µl of ABS produced no antibacterial effect to any clinical isolates and standard strains. In the broth micro dilution method, ABS did not dissolve in Mueller–Hinton broth (MHB) [15]. For this reason, antimicrobial effect of ABS was tested by agar well diffusion technique [16].

All clinical isolates were cultured on blood-agar plates and the plates were incubated for 24 h at 37 °C. After incubation, bacterial concentration was adjusted to McFarland no standard 0.5 and swabbed on Muller–Hinton agar plates at three directions according to CLSI [14]. After swabbing the bacteria, we made 0.8 cm well in the middle of the medium. Afterwards, 100 µl ABS solutions were put into these wells.

2.4. Determination of antimicrobial activity of ABS

All plates were incubated for 24 h at 37 °C. After overnight incubation, bacterial inhibition was determined by measurement of the diameter of inhibition zone.

3. Results and discussion

The antimicrobial activity of ABS was investigated on 102 clinical isolates and standard strains by agar well diffusion assay (Table 2). This folk medicine was active against all bacteria investigated. Inhibitory zones for all isolates were determined as 10–18 mm (Table 2). ABS produced an inhibitory zone with a mean diameter of 14 mm with both Gram negative and Gram positive bacteria. ABS was especially active against *A. baumannii* (mean; 15 mm), MRSA (mean; 15 mm) and ESBL (+) *E. coli* (mean; 15 mm).

Medicinal plants have been used for ages in the treatment of diseases [17]. In recent years, herbal medicines have increasingly been using to treat infections difficult to manage [18]. Plants are known to produce certain chemicals which are naturally toxic to bacteria, and a large body of literature has validated the antimicrobial activity of plant extracts, showing great potential especially against multidrug resistant bacteria [19,20].

ABS has been approved in Turkey for the management of external haemorrhage and bleeding at dental surgery [2]. The effect of ABS on homeostasis has been reported [1]. Although the antibacterial activity of all ingredients of ABS has been investigated, the clinical relevance of the bacterial strains is not clear. Our investigation has provided clinically relevant data, since all the microorganisms tested were isolated from infected foci of hospitalized patients (Table 1). Inhibitory zone for all isolates were determined as 10–18 mm. Mean diameters of the zones for Gram negative and positive bacteria were found 14 mm. Plant extracts are generally much more active on Gram (+) bacteria [21], and the activity of ABS on Gram (–) bacteria might be due to the synergistic combination of the single extracts. Of special relevance is the activity against *P. aeruginosa*, *A. baumannii*, and ESBL (+) *E. coli*, with 12, 15 and 15 mm of inhibition diameter, respectively. These bacteria are known to be the most frequent causes of nosocomial antibiotic-resistant infections.

Table 2

Antibacterial activity of Ankaferd Blood Stopper® for microorganisms with agar well diffusion method

Microorganisms	Number of isolates	Mean diameter of inhibitory zone (mm) (min–max)
MRSA	9	15 (13–18)
<i>Enterococcus</i> spp.	9	14 (10–15)
VRE	10	13 (12–14)
MSSA	7	14 (13–16)
MRKNS	9	14 (12–15)
<i>Enterobacter</i> spp.	10	12 (10–14)
<i>P. aeruginosa</i>	9	12 (12–14)
<i>A. baumannii</i>	10	15 (14–17)
ESBL (+) <i>E. coli</i>	9	15 (11–17)
ESBL (+) <i>K. pneumonia</i>	10	14 (12–16)
<i>S. maltophilia</i>	10	14 (13–18)
MRSA ATCC 43300	1	18
MSSA ATCC 25923	1	14
<i>P. aeruginosa</i> ATCC 27853	1	12
<i>E. coli</i> ATCC 35218	1	12

MRSA: Methicillin-resistant *Staphylococcus aureus*, VRE: Vancomycin-resistant enterococci, MSSA: Methicillin-sensitive *Staphylococcus aureus*, MRKNS: Methicillin-resistant coagulase negative staphylococci.

The most striking result of our investigation is the documentation of a remarkable antimicrobial activity of ABS against different groups of multidrug resistant bacteria. The very promising results we have obtained provide a rationale for assessing the activity of ABS on infected wounds.

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