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Antimicrobial Activity of Ankaferd Blood Stopper® Against Nosocomial Bacterial Pathogens

Research Article

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Abstract: The aim of this study is to investigate the in vitro antimicrobial activity of Ankaferd Blood Stopper® against methicillin-resistant Staphylococcus aureus (MRSA), *Enterococcus* species, *Escherichia coli*, *Pseudomonas* species, *Acinetobacter* species and *Klebsiella* species of nosocomial origin. Ankaferd inhibited growth in 72.4% to 100% of the bacteria tested, depending on the type of the isolate. As a result, it can be stated that Ankaferd inhibits the in vitro growth of nosocomial bacteria. This is a novel, important finding since severe hospital infections coexist with many hemostatic disorders, and the use of Ankaferd may increase hemostatic potential in such clinical conditions.

Keywords: *Ankaferd Blood Stopper® • Antibacterial activity • Nosocomial infection • Hemostatic agent*

1. Introduction

Ankaferd Blood Stopper is a standardized herbal extract obtained from five different plants: *Thymus vulgaris* (5.0 g/100 ml), *Glycyrrhiza glabra* (7.0 g/100 ml), *Vitis vinifera* (8.0 g/100 ml), *Alpinia officinarum* (7.0 g/100 ml), and *Urtica dioica* (6.0 g/100 ml) [1]. The extract has been approved in Turkey for the clinical management of bleeding resulting from external and dental surgery [2]. Ankaferd also has been used to manage hemorrhage in difficult clinical conditions [3-8]. Ankaferd represents a unique hemostatic effect by promoting the very rapid (<1 sec) formation of a protein network, which acts as an anchor for vital physiological erythrocyte aggregation and covers the classical cascade model of the clotting system without independently acting on coagulation factors and platelets [1,8].

Ankaferd was analysed via two-dimensional gel electrophoresis and mass spectrometer (MALDI-TOF). Herbal proteins that composed Ankaferd were identified. These were NADP-dependent malic enzyme, ribulose biphosphatecarbocsilase large chain, maturase K, ATP synthase beta subunit, ATP synthase alpha subunit, chalcon flavon isomerase-1, chalcon flavon isomerase-2, and actin depolimerisation factor. Some human-protein-like proteins that are important in coagulation, such as ATP synthase, mucin 16, helezonal bundle transporter protein-141, were also identified [9].

Proteomic analysis [9], unique effects on critical transcription factors [10], and in vitro anti-infectious and anti-cancer effects [11-14] suggested that Ankaferd may affect the pathobiological course of disease in tissue, in addition to its unique action on hemostasis.

Antibiotic-resistant bacteria represent a great challenge in the treatment of nosocomial infections

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Table 1. The inhibition zone diameters of Ankaferd and control antibiotics against clinical isolates.

	Ankaferd		Control antibiotics	
	≤10mm	>10mm	≤10mm	>10mm
Bacteria	n (%)	N (%)	n (%)	n (%)
Methicillin-resistant *Staphyloccocus aureus* (MRSA)	2 (6.7)	28 (93.3)	4 (13.3)	26 (86.7)
Enterococcus species	0 (0)	30 (100.0)	3 (10.0)	27 (90.0)
Escherichia coli	2 (6.7)	28 (93.3)	0 (0)	30(100.0)
Klebsiella species	8 (26.6)	22 (73.3)	3 (10.0)	27 (90.0)
Acinetobacter species	0 (0)	30 (100.0)	14 (46.7)	16 (53.3)
Pseudomonas species	1 (3.3)	29 (96.7)	1 (3.3)	29 (96.7)

[15-22]. Infection of wounds, especially with nosocomial bacteria, is an important complication that impairs wound-healing and increases the duration of hospitalization and the use of broad-spectrum antimicrobials.

The aim of this study was to evaluate in vitro antibacterial activity of Ankaferd Blood Stopper® against methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus* species, *E. coli, Pseudomonas* species, *Acinetobacter* species, and *Klebsiella* species, which are the most frequently isolated agents of nosocomial infections [1].

2. Material and Methods

2.1. Ankaferd Blood Stopper

The standardized vials of the Ankaferd Blood Stopper (ABS, one vial of 100 ml; patent number 2007-0-114485) used in the experiments were supplied free of charge by Ankaferd Drug Inc., Istanbul, Turkey.

2.2. Bacterial isolates

Clinical isolates of MRSA, *E. coli, and Enterococcus*, *Pseudomonas, Acinetobacter*, and *Klebsiella* species that were proved to be causative agents of hospital infection were included in the study. A total of 180 isolates (30 isolates of each genus) were tested. The isolates were recovered from the clinical samples taken from adult inpatients at Hacettepe University, Faculty of Medicine, Adult Hospital during the period 2007-2008. The identification of the bacteria was performed with the automated identification system (Phoenix, Becton-Dickinson, USA). MRSA and vancomycin-resistant enterococcus (VRE) isolates were previously identified by standard antibiotic susceptibility testing, according to guidelines of the Clinical and Laboratory Standards Institute (CLSI) [23].

2.3. Antibacterial activity testing

The agar well diffusion method was used for testing the antibacterial activity of Ankaferd [24,25]. Stock isolates that were grown on blood agar were subcultured onto Trypticase soy broth. The broth cultures were incubated at 37°C for 3 to 6 hours in order for the bacteria to reach the log phase. The Trypticase soy broth cultures were adjusted to the 0.5 McFarland turbidity standard, and 100 µl were taken from the suspension and added to the semi-solid media (0.7% nutrient agar) that was melted to the liquid form. The cultures were "flood-inoculated" onto the surface of Mueller-Hinton agar plates. When the semisolid nutrient agar was solidified, wells, 8 mm in diameter, were cut into the media with a sterile cork-borer. Ankaferd (100 µl) was added to one of the wells, while a control antibiotic (100 µl) was added to the other well. Vancomycin was used as the control antibiotic for the MRSA and *Enterococcus* species isolates, and imipenem was used for *E. coli, and Pseudomonas, Acinetobacter* and *Klebsiella* species isolates. All the plates were incubated at 37°C under normal atmospheric conditions for 18 hours. The antibacterial activity of Ankaferd and the control antibiotic was determined by measuring the zone of inhibition around the wells. When a zone of inhibition higher than 10 mm was obtained, Ankaferd was interpreted as having inhibitory activity against the clinical isolate tested. When the inhibition zone was less than 10 mm, it was accepted that Ankaferd had no inhibitory activity against that clinical isolate.

3. Results

The antimicrobial activity of ABS was tested in a total of 180 bacterial strains. The inhibition zone diameters obtained by Ankaferd and control antibiotics are shown in Table 1. Between 72.4% and 100% of the isolates were inhibited by Ankaferd. The overall ratio of isolates inhibited by Ankaferd was 92.6%. Ankaferd was effective against all *Enterococcus* and *Acinetobacter*

isolates. This result was of importance since 90% of *Enterococcus* species was inhibited by the control antibiotic vancomycin, and 53.3% of *Acinetobacter* was inhibited by imipenem. The activity of Ankaferd was clearly seen among the isolates of *Acinetobacter*. The zone of inhibition for imipenem was <10 mm in 46.7% of *Acinetobacter* isolates, whereas the zone of inhibition for Ankaferd was ≥10 mm in all isolates of *Acinetobacter*. Similarly, Ankaferd was shown to be effective against isolates of VRE. One isolate that was resistant to vancomycin exhibited a zone of inhibition with Ankaferd 13 mm in diameter, and two other isolates that were vancomycin-resistant had zones 16 mm in diameter.

Ankaferd also exhibited antibacterial activity against clinical isolates of MRSA. Four MRSA isolates that showed decreased susceptibility to vancomycin had zones of inhibition with Ankaferd at a diameter of ≥10 mm. Although this method of testing vancomycin susceptibility is not standard, this observation suggests that Ankaferd had higher diameters of inhibition zones for some of the MRSA isolates. When *Klebsiella* isolates were considered, we concluded that the least activity was observed with these isolates, since only 73.3% seemed to be inhibited by Ankaferd. Ankaferd exhibited inhibitory activity similar to the control antibiotic against *Pseudomonas* species and *E. coli* isolates.

4. Discussion

On the basis of the results of this study, Ankaferd, in addition to its hemostatic activity, may inhibit the growth of bacteria. Anti-infectious activity of Ankaferd may represent an advantage over its current clinical use in hemostasis, since it inhibits the growth of bacteria in areas where it is used mainly for its hemostatic activity, such as in traumatic infected wounds. We tested the most common agents responsible for hospital infections worldwide. Ankaferd was found to be effective in 92.6% of the isolates. In the study by Tasdelen-Fisgin et al., Ankaferd's antimicrobial activity was tested against 102 clinical isolates [14]. The isolates included *Acinetobacter baumannii, E. coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Enterobacter* species, *Stenotrophomonas maltophilia,* MRSA, methicillin-resistant coagulase-negative *Staphylococcus,* vancomycin-susceptible *Enterococcus*, and VRE. These observers reported that Ankaferd was active against all these isolates, with zones of inhibition that were within the range of 10 to 18 mm; these results were in accordance with our study. Anti-bacterial activities of Ankaferd against several gram-positive and gram-negative food and human pathogens were also reported by Akkoc, et al. [11].

In this preliminary study, in vitro antibacterial activity of Ankaferd was demonstrated against the most frequently encountered agents of nosocomial infections. Further studies that include a high number of isolates and in vivo clinical response should be planned. Ankaferd's antibacterial activity, especially against MRSA, VRE, and imipenem-resistant *Acinetobacter* isolates, cannot be neglected. Since infection and numerous hemostatic disorders could coexist, the use of Ankaferd may have therapeutic potential in such clinical settings. In conclusion, Ankaferd, initially introduced as a hemostatic agent, also exhibits antibacterial activity, and further studies are needed to evaluate the application of Ankaferd as an alternative antibacterial agent of natural origin.

The mechanism of action of Ankaferd on bacterial pathogens should be investigated in future studies. Ankaferd is a hemostatic agent with pleiotropic effects [1,3-14,26-28]. Ankaferd has been shown to promote the formation of an encapsulated protein mesh, which acts as an anchor for erythrocyte aggregation, with in vitro studies paralleling those in vivo [1,8,14,26-28]. Ankaferd also has significant anti-infective biologic activity against a wide variety of pathogens [11,14]. The unique protein library of Ankaferd upregulates critical transcription factors [9,10]. On the basis of the observations in this report, hemostatic action of ABS is associated with an inhibitory effect on nosocomial pathogens.

Hospitalized patients may experience various life-threatening infection-induced hemorrhages, most frequently due to coagulation defects such as thrombocytopenia and disseminated intravascular coagulation [3,29-31]. Ankaferd-induced formation of the protein network with vital erythroid aggregation includes the entire physiologic hemostatic process [1,8]. Mainly, there are distinct important components of the Ankaferd-induced hemostatic network. Vital erythroid aggregation takes place in association with the spectrin and ankyrin receptors on the surface of red blood cells. Those proteins and the required ATP bioenergy are included in the protein library of Ankaferd [9]. Ankaferd also upregulates the GATA (FOG) transcription system affecting erythroid function [10]. Urotensin II is also an essential component of Ankaferd, and it represents the link between injured vascular endothelium, adhesive proteins, and active erythroid cells [9]. Those concepts have been developed via MALDI-TOF proteomic molecular analyses, cytometric arrays, transcription analysis, and SEM ultrastructural examinations, as well as by numerous interactive investigations between basic and clinical research facilities [1,3,8-10,12-14,26-28]. Therefore, ABS could be used effectively both in individuals with normal hemostatic parameters and in

patients with deficient primary hemostasis or secondary hemostasis, or both. In vitro data on the anti-infectivity profile of Ankaferd and bleeding control in the setting of gastrointestinal disorders [5-7] and mediastinal bleeding [3,4] shed further light on the upcoming controlled trials.

Further investigations of the exciting features of Ankaferd as a powerful hemostatic agent are in progress. Our observations on the effect of Ankaferd on infections may open new avenues for its future development.

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