

IN VITRO ANTI-BACTERIAL ACTIVITIES OF ANKAFERD MEDICINAL PLANT EXTRACT

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ABSTRACT

The medicinal value of plants lies in some chemical substrates that produce a definitive physiological action on the human body. *Ankaferd* comprises a standardized mixture of the plants *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum* and *Urtica dioica*. The aim of this study is to assess *in vitro* antimicrobial activities of *Ankaferd* medicinal plant extract. The antimicrobial activity assay was performed by agar well diffusion to assess the antagonistic activity of *Ankaferd* against 26 indicator strains including human pathogens and food spoilage, Gram-negative and Gram-positive bacteria. *Ankaferd* was found to be active against all bacteria tested while nisin, the only commercial bacteriocin for food preservation, was inactive against Gram-negative indicator strains. Besides a high inhibitory activity against Gram-positive and Gram-negative bacteria, including human pathogens and food spoilage bacteria, *Ankaferd* was found to be more stable than nisin in different heat and enzyme treatments. Antibacterial activity of *Ankaferd* can be extended to extreme environmental conditions such as potential use of the preparation for the therapy of infectious diseases and preservation of different type foods from food-born pathogens or food spoilage bacteria. The demonstration of the anti-infective properties of *Ankaferd* adds a new value to its haemostatic effect in the healing of infected hemorrhagic wounds, as well as opening new avenues for its potential use in anti-infective actions and food preservation.

KEY WORDS

Ankaferd; *Thymus vulgaris*; *Glycyrrhiza glabra*; *Vitis vinifera*; *Alpinia officinarum*; *Urtica dioica*; Herbal remedies; Infection; Haemostasis

INTRODUCTION

The medicinal value of plants lies in some chemical substrates that produce a definitive physiological action on the human body. The use of plant extracts and phytochemicals, with established antimicrobial properties, could be of great significance in preventive and/or therapeutic approaches. The most important antimicrobial compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [1-4]. The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies [5,6]. Contrary to synthetic drugs, antimicrobials of plant origin usually are not associated with many side effects and have an enormous anti-infective potential in numerous infectious diseases. Based on *World Health Organization (WHO)* reports, more than 80% of the World population relies on traditional medicine for their primary healthcare needs [2,7,8].

Ankaferd is a unique folkloric medicinal plant extract, which has historically been used in Turkish traditional medicine as a haemostatic agent. *Ankaferd* comprises a standardized mixture of the plants *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum* and *Urtica dioica*. The basic mechanism of action for pharmacological preparation of *Ankaferd* Blood Stopper[®] is the formation of an encapsulated protein network that provides focal points for erythrocyte aggregation. Exposure to *Ankaferd* seems to provide a tissue oxygenation as well as a physiological haemostatic process without affecting any individual clotting factor [9]. The aim of this study is to assess *in vitro* antimicrobial activities of *Ankaferd* medicinal plant extract. Elucidation of the anti-infective properties of *Ankaferd* adds a new value to its haemostatic effect in the healing of infected hemorrhagic wounds, as well as opening new avenues for its potential use in anti-infective actions and food preservation.

MATERIALS AND METHODS

Bacterial strains

The bacteria used in this study were propagated in appropriate media as indicated in Table 1. Bacterial stocks were stored at – 80 °C in their respective broths supplemented with 30 % glycerol.

Antimicrobial activity assay

The antimicrobial activity assay was performed by agar well diffusion method [10]. Wells were prepared in the plates with help of a cork-borer (0.85 cm). 100 µL of the test compound was introduced into well. The plates were incubated overnight at appropriate incubation temperatures for indicator strains. Microbial growth was determined by measuring the diameter of zone of inhibition.

The effect of heat and enzymes on medicinal plant extract, Ankaferd

The samples of *Ankaferd* (one vial of 100 ml) were provided by *Ankaferd Drug Inc.*, (*Ankaferd* patent number 2007-0-114485; Trend Teknoloji İlaç AS, Istanbul, Turkey, www.ankaferd.com). To determine the effect of heat on *Ankaferd*, test samples were heated 80 °C, 90 °C, 100 °C and 121 °C for 15 min. *Ankaferd* was also treated with the following enzymes at final concentration of 1 mg.mL⁻¹: trypsin (pH 7.0, Sigma, Deinsenhofen, Germany), α-chymotrypsin (pH 7.0, Sigma), proteinase K (pH 7.0, Sigma), pepsin (pH 3.0, Merck, Darmstadt, Germany), α-amylase (pH 7.0, Sigma), lipase (pH 7.0, Sigma), catalase (pH 7.0, Sigma) and lysozyme (pH 7.0, Sigma). Following incubation at 37 °C for 2 h, enzyme activity was terminated by heating at 100 °C for 5 min. Untreated samples were used as controls [11].

RESULTS AND DISCUSSION

Antagonistic Activity

Well diffusion assay was performed to assess the antagonistic activity of *Ankaferd* against 26 indicator strains including human pathogens and food spoilage, Gram-negative and Gram-positive bacteria. The folkloric medicinal extract, *Ankaferd*, was found to be active against all bacteria tested while nisin, the only commercial bacteriocin for food preservation, was inactive against Gram-negative indicator strains (Table 1 and Figure 1).

Amongst the Gram-positive and Gram-negative bacteria, Gram-positive bacterial strains were reported more susceptible to the medicinal plant extracts as compared to gram-negative bacteria [12,13]. *Ankaferd* showed a high inhibitory activity for both Gram-negative and Gram-positive bacteria. This unique property of the *Ankaferd* medicinal plant extract pointed out its antimicrobial potential as a drug and foods additive.

Effect of Heat and Enzymes on Ankaferd

The enzymatic activity and heat stability of the *Ankaferd* compared to that of nisin (Sigma Chem. Co. USA) was used an experimental control. The *Ankaferd* medicinal extract, was found to be heat stable and did not affected by any enzymes tested whereas control bacteriocin, nisin, activity was completely lost after α -chymotrypsin and proteinase K treatment (Table 2, Figure 2 and Figure 3).

Besides a broad-spectrum activity against gram-positive and gram-negative bacteria, including human pathogens and food spoilage bacteria, *Ankaferd* was found to be more stable than nisin in different heat and enzyme treatments. The results presented here showed that the antibacterial activity of *Ankaferd* can be proceed extreme environmental conditions such as potential use of the preparation for the therapy of infectious diseases and preservation of different type foods from food-born pathogens or food spoilage bacteria. When added to plasma or serum, *Ankaferd* induces the very rapid formation of a protein network and erythrocyte aggregation [9]. Sequelae of severe infection and sepsis may cause accelerated clearance of erythrocytes from circulating blood and the exposure of erythrocytes to pathogens can induce eryptosis [14]. Furthermore, alterations in red blood cell function can contribute to pathological changes in microcirculatory blood flow and cellular dysoxia in sepsis [15]. In a recent study, the haemostatic mechanism-of-action of the *Ankaferd* Blood Stopper[®], have been investigated [9]. *Ankaferd* stimulated the formation of an encapsulated protein network that provides focal points for erythrocyte aggregation [9]. Since infection and bleeding coexist in a variety of disorders [16-18], *Ankaferd* has the therapeutic potential to be used for the management of haemorrhage in these difficult clinical conditions. Haemostatic process and bacterial infections have significant cross-talks regarding biological basis [19-23] and clinical grounds [24-26]. Our present results indicated that future studies on *Ankaferd* should focus on not only the haemostatic actions but also anti-infective properties of this unique medicinal plant extract in health and disease.

Table 1. Inhibitory spectrum of *Ankaferd* medicinal plant extract

Indicator strain	Inhibitory Activity*		Source**	Media***
	<i>Ankaferd</i>	Nisin(1000IU/mL)		
<i>L. lactis</i> subsp. <i>lactis</i> SIK-83 (nisin producer)	++	-	NHL	M17
<i>L. lactis</i> subsp. <i>lactis</i> ATCC7962 (nisin producer)	++	-	NHL	M17
<i>L. lactis</i> subsp. <i>lactis</i> LMG2908 (nisin producer)	++	-	NHL	M17
<i>Micrococcus luteus</i> NCIMB8166	+++	+++	AUFF	NB
<i>Bifidobacter bifidum</i> CHL17	+++	++	NHL	MRS
<i>Bifidobacter longum</i> CHL21	+++	++	NHL	MRS
<i>Lactobacillus sake</i> NCDO2714	++	++	NHL	MRS
<i>Lactobacillus plantarum</i> LMG2003	++	++	NHL	MRS
<i>Leuconostoc carnosum</i> DSM5576	++	++	NHL	MRS
<i>Enterococcus faecium</i>	++	++	AUFF	MRS

Table 1. Inhibitory spectrum of *Ankaferd* medicinal plant extract (continue)

Indicator strain	Inhibitory Activity*		Source**	Media***
	<i>Ankaferd</i>	Nisin(1000IU/mL)		
<i>Enterococcus faecalis</i> LMG2602	++	++	NHL	MRS
<i>Staphylococcus aureus</i> ATCC6538	+++	++	NHL	NB
<i>Staphylococcus carnosus</i> MC1B	+++	++	NHL	NB
<i>Clostridium tybutyricum</i>	+++	++	NHL	RCM
<i>Clostridium sporogenes</i>	+++	++	NHL	RCM
<i>Bacillus subtilis</i> 12	++	+++	NHL	NB
<i>Bacillus licheniformis</i> 40	++	+++	NHL	NB
<i>Bacillus cereus</i> LMG2732	++	+++	NHL	NB
<i>Pseudomonas fluorescens</i> P1	+++	-	NHL	NB
<i>Pseudomonas aureginosa</i> ATCC15442	+++	-	NHL	NB
<i>Escherichia coli</i> CFA1	+++	-	NHL	LB

Table 1. Inhibitory spectrum of *Ankaferd* medicinal plant extract (continue)

Indicator strain	Inhibitory Activity*		Source**	Media***
	<i>Ankaferd</i>	Nisin(1000IU/mL)		
<i>Salmonella enterica</i> Typhimurium	+++	-	NHL	LB
<i>Klepsiella pneumonia</i>	++	-	AUFF	NB
<i>Pediococcus pentosaceus</i> LMG2001	++	++	NHL	MRS
<i>Listeria innocua</i> 2813	++	+++	NHL	NB
<i>Listeria monocytogenes</i> ATCC15313	++	+++	NHL	NB

* - , no inhibition zone; ++, 5mm ≤ inhibition zone ≤ 10 mm, > 10 mm inhibition zone

** NHL, Agricultural University of Norway, As, Norway; AUFF; Faculty of Science, University of Ankara, Ankara, Turkey

*** MRS, de Man-Rogosa-Sharpe; LB, Luria-Bertani; NB; Nutrient broth, RCM; Reinforced clostridial medium



Figure 1. Inhibition zones of *Ankaferd* against *S. enterica typhimurium*

Table 2. Effect of enzymes and heat treatment of the *Ankaferd* medicinal plant extract

Treatment	Inhibitory Activity*	
	<i>Ankaferd</i>	Nisin (1000 IU/mL)
Enzymes		
Trypsin (Sigma, No. T8658)	+++	+++
α -chymotrypsin (Sigma, No. C-6423)	+++	-
Proteinaz K (Sigma, No. P-6390)	+++	-
Pepsin (Merck, No. 7147)	+++	+++
α -amylase (Sigma, typ VII A)	+++	+++
Lipase (Sigma, No. L17714)	+++	+++
Catalase (Sigma, No. C-10)	+++	+++
Lysozyme (Sigma, No. L7651)	+++	+++
Heat		
15 min at 80 °C	+++	+++
15 min at 90 °C	+++	+++
15 min at 100 °C	+++	++
15 min at 121 °C	+++	+

* Inhibitory activity was determined by using the indicator strain *Micrococcus luteus* NCIMB8166

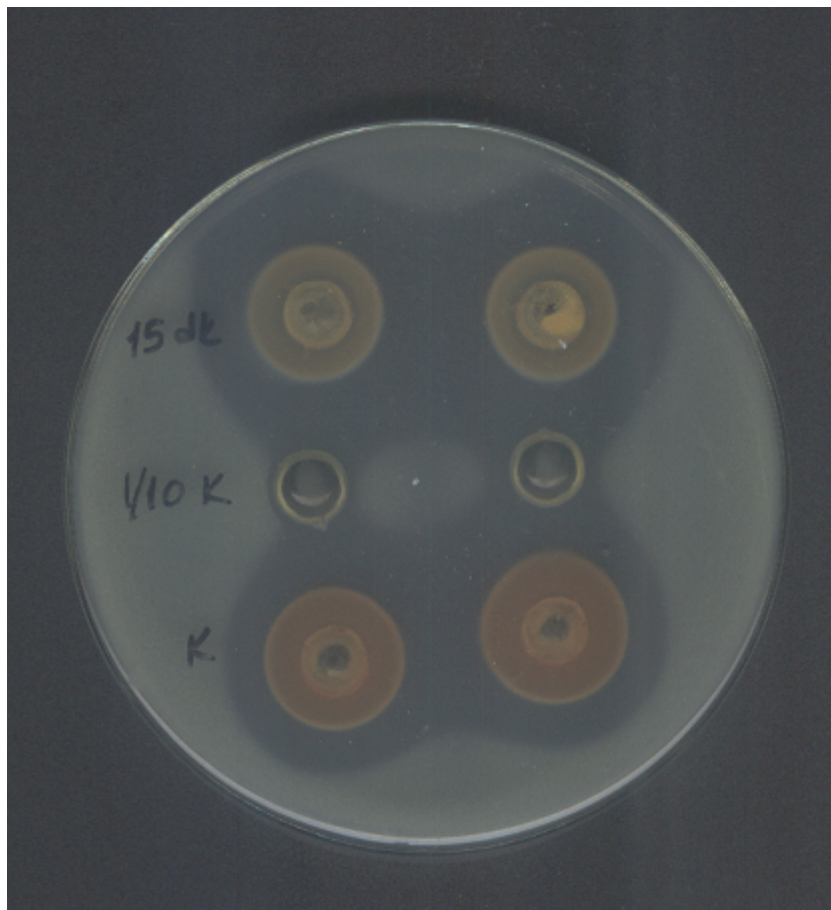


Figure 2. Inhibition zones of *Ankaferd* against *Micrococcus luteus* NCIMB8166 after treatment of 15 min at 121 °C

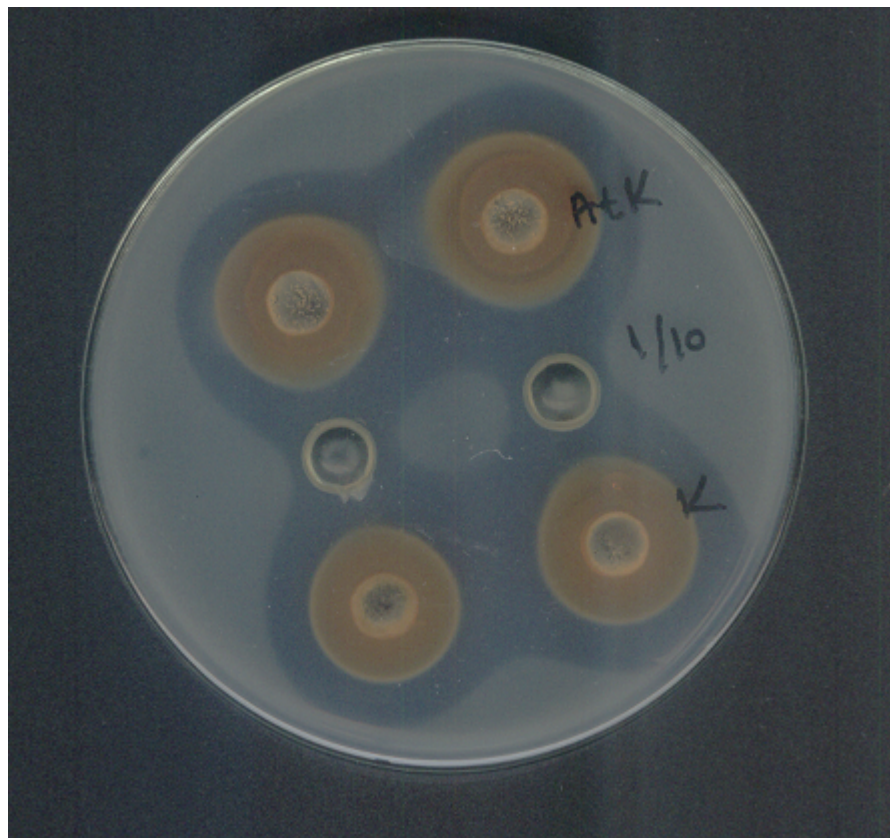


Figure 3. Inhibition zones of *Ankaferd* against *Micrococcus luteus* NCIMB8166 after proteinase K treatment

Conflicts of interest

Ankaferd is a traditional folkloric medicinal plant extract that has been developed by HC Firat.

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