

## STUDY ON ANTIBACTERIAL EFFECT OF SOME ESSENTIAL OILS ON *S.EQUI* AND *S.ZOOEPIDEMICUS*

By

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### ABSTRACT

In the present study there were 2 *S.equi* isolates and 2 *S.zooepidemicus* isolates recovered from horses suffered clinically from strangles symptoms. The isolates confirmed serologically by using specific antisera and also molecularly by using PCR. Three essential oils (cinnamon, anise and oreganium) were tested for their antimicrobial activity against *S.equi* and *S.zooepidemicus* . Results were recorded that cinnamon oil completely inhibited the growth of all the tested bacterial isolates (100%) at a concentration of 3% and (50%) at a concentration of 2%, on contrary, 1% concentration had no effect on all the tested isolates , while anise oil did not show any growth inhibitory effect at any concentration used in the present study (1%, 2%, 3%, 4%, 5% and 6%), In other hand oreganium oil exerted a strong growth inhibitory effect on *Streptococcus* isolates at concentration of 1% (100%). while at 0.5% concentration has no effect.

### **Key words :**

(*Streptococcus*, Essential oils, Antibacterial effect ).

### INTRODUCTION

Strangles is an important infectious disease affecting horses. It is caused by *Streptococcus equi* subspecies *equi*, a member of the Lancefield C group streptococci, and is characterized by an acute, febrile, suppurative, lymphadenitis (Harrington *et al.*, 2000). Horses suffering from strangles have mucopurulent nasal discharge and abscesses, which may often burst and exude. Affected populations present high morbidity levels and infection may cause chronic illness or even death. Strangles is very contagious, especially with foals, spreading easily from horse to horse and often leading to large outbreaks (Sweeney *et al.*, 1996). Antibiotic resistance is a serious and growing phenomenon in contemporary medicine and has emerged

as one of the pre-eminent public health concerns in 21st century. World Health Organization's 2014 report on global surveillance of antimicrobial resistance states that "antibiotic resistance is a serious threat and no longer a prediction for the future; it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country". This jeopardizes the treatment of common infections in the community and hospitals (WHO, 2014). It has also been predicted by several authors that, the next pandemic will not be of some specific disease but due to ineffectiveness of available drugs to cure even small cuts and wounds. Herbal medicine has always been a part of Indian culture and gaining popularity due to toxicity and side effects of allopathic medicines. This led to sudden increase in the number of herbal drug manufactures in India (Agarwal *et al.*, 2004). It's reported that more than 500 Indian traditional communities use about 800 plant species for curing different diseases among 20,000 medicinal plant species that are available in the country (Kamboj *.,2000*). The burning question is now, if non-judicious use of antibiotics may lead to emergence and spread of ADR why it may not happen to herbal antimicrobials? In recent past, a few reports have already documented about prevalence of herbal antimicrobial drug resistance (HADR) in environmental and clinical strains of bacteria. However, either the acceptance of the existence of HADR (probably due to looking those as last resources) or due to poor understanding of HADR. So the aims of the present study were detection of antibacterial effect of (Cinnamon, Anise, and Oreganium) essential oils on *S.equi* and *S.zooepidemicus*.

## **MATERIAL AND METHODS**

### **Preparation of the bacterial suspension.**

A bacterial suspension ( $1.5 \times 10^8$ CFU/ml) was prepared using physiological saline matching McFarland's opacity tube No. 0.5.

### **Preparation of oils.**

- Herbal oils as cinnamon, anise and oreganium.
- All the tested herbal oils were sterilized by filtration using millipore cellulose filter membrane (0.45  $\mu$ m pore diameter) and were mixed with medium at concentrations of 1, 2, and 3% for cinnamon oil, 1, 2, 3, 4, 5 and 6% for anise oil and 0.5 and 1% for oreganium oil.

### **Evaluation of the effect of herbal oils on the growth of *S.equi* and *S.zooepidemicus*.**

According to the method of Jeff-Agboola *et al.* (2012) the antibacterial activity of cinnamon, anise and oreganium against recovered isolates were tested. Briefly, the tested bacteria were

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grown on tryptone soya agar at 37°C for 24 hr, then cells were suspended in physiological saline (0.9% NaCl), and the suspension was adjusted to  $1 \times 10^8$  CFU. Tryptone soya agar was prepared and autoclaved at 121°C for 15 min. and kept at 55°C and then the tested oils were mixed with TSA according to the tested concentrations. The oil-agar medium (10 ml) was then poured into sterile petri dishes and was solidified. Equal amounts of the bacterial suspensions were inoculated and spread onto the agar plates. The plates were then incubated at 37°C for 24 - 48hr.

### RESULTS

Three essential oils (cinnamon, anise and oreganium) were tested for their antimicrobial activity against *S.equi* and *S.zooepidemicus*. Results were recorded in (Tables 1, 2).

#### **Detection of the antibacterial effect of Cinnamon oil on *Streptococcus* isolates.**

As shown in (Table 1) cinnamon oil completely inhibited the growth of all the tested bacterial isolates (100%) at a concentration of 3% and (50%) at a concentration of 2%, on contrary, 1% concentration had no effect on all the tested isolates.

**Table (1):** Detection the antibacterial effect of Cinnamon on *Streptococcus* isolates.

Bacterial isolates	No. of tested isolates	Effect of Cinnamon oil concentration on isolates					
		1%		2%		3%	
		NO.	%	NO.	%	No.	%
<i>S.equi</i>	2	0	0	1	50	2	100
<i>S.zooepidemicus</i>	2	0	0	1	50	2	100

%; was calculated according to number (NO.) of tested bacterial isolates.

#### **Detection the antibacterial effect of anise oil on *Streptococcus* isolates.**

Anise oil did not show any growth inhibitory effect at any concentration used in the present study (1%, 2%, 3%, 4%, 5% and 6%).

#### **Detection of the antibacterial effect of oreganium oil on *Streptococcus* isolates.**

As shown in (Table 2) Oreganium oil exerted a strong growth inhibitory effect on *Streptococcus isolates* at concentration of 1% (100%). while at 0.5% concentration has no effect.

**Table (2):** Detection the antibacterial effect of Oreganium oil *Streptococcus* isolates.

Bacterial isolates	No. of tested isolates	Effect of Organium oil concentration			
		0.5%		1%	
		NO.	%	NO.	%
<i>S.equi</i>	2	0	0	2	100
<i>S.zooepidemicus</i>	2	0	0	2	100

**%:** was calculated according to number (NO.) of tested bacterial isolates.

## DISCUSSION

Antimicrobial drug resistance (ADR) hampers the control of infectious diseases and has potential to threaten health security, damage trade and economies but it is difficult to think of “the world without antibiotics”. It may be a deadly situation because the routine surgery, cancer treatments, organ transplants etc. become just impossible without antibiotics. So, antibiotics need to be saved for certain therapeutic interventions. Development of ADR is a natural phenomenon (**Fleming A., 1929**). However, certain human actions accelerate the emergence and spread of ADR. Inappropriate therapeutic use of antimicrobial drugs, and use in agriculture, fish, poultry and animal farming, favours the emergence and selection of resistant strains. Besides, poor infection prevention and control practices further contribute for emergence and spread of ADR. Eminent organizations like WHO, World Organization for Animal Health (OIE) and Food and Agriculture Organization (FAO) of the United Nations have collaborated to promote best practices to avoid the emergence and spread of antibacterial resistance. All attempts are in progress to promote optimal use of antibiotics both in humans and animals to address problem of growing AMR. Most of the pathogenic bacteria have developed resistance to modern antibiotics as a result of which multi drug resistance among bacteria is being evidenced. The world running out of antibiotics and could not add any new group of antibiotics since last three decades. At the same time, there is no potential antibiotic in pipeline for release in near future. As a result, research in alternative medicine has begun and one such alternative is use of herbal drugs to treat infections. Since ancient times, herbs and their essential oils are known for their varying degrees of antimicrobial activity. Due to immense biodiversity, India is a vast repository of medicinal plants that are used in traditional medical treatments (**Chopra et al., 2002**). Almost, 70% modern medicines in India are

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derived from natural products and the various indigenous systems of India such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments (Rabe *et al.*, 1994). It has been reported that volatile oils from plants have analgesic, antibacterial, deodorizing, febrifuge, fungicidal, antiseptic, antidepressant, astringent, diuretic, galactagogue, insecticidal, antipyretic, antimicrobial and sedative properties. In the present study three essential oils (cinnamon, anise and oreganium) were tested for their antimicrobial activity against *S.equi* and *S.zooepidemicus*. Results were recorded that cinnamon oil completely inhibited the growth of all the tested bacterial isolates (100%) at a concentration of 3% and (50%) at a concentration of 2%, on contrary, 1% concentration had no effect on all the tested isolates, while anise oil did not show any growth inhibitory effect at any concentration used in the present study (1%, 2%, 3%, 4%, 5% and 6%), In other hand Oreganium oil exerted a strong growth inhibitory effect on *Streptococcus* isolates at concentration of 1% (100%). while at 0.5% concentration has no effect. Among herbal preparations, essential oils of several medicinal plants are often shown to possess antimicrobial activities. Among all the oils, the essential oil of cinnamon has been found to be the most effective, followed by the essential oil of oregano and thyme (the active ingredient in latter two plants is caracole). The demand for the herbal drugs has increased in recent times, as many plants or herbs are scientifically proven to contain bioactive compound (s) and as alternatives to harmful synthetic drugs that cause side effects to biological system and environment (Evans ., 2008).

### CONCLUSION

Our study concluded that there are recent large-scale evaluation of herbal medicine for control of antibiotic resistance between equine streptococcal isolates submitted to a university diagnostic lab.

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