THE EFFECT OF ETHANOLIC HERBAL EXTRACT ON MICROORGANISMS

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Abstract: Plants produce a wide range of organic compounds including tannins, organic acids, essential oils and micronutrients, which can inhibit growth, reproduction and other life processes. These compounds can be found in various plants. Extracts from plants can be used for food preservation and for human or animal healing. In this study the effect of ethanolic extract of *Cannabis sativa L.*, *Silybum marianum* and *Hippophae rhamnoides* was tested on *Escherichia coli* (CCM 7929), *Enterococcus faecalis* (CCM 4224), *Lactobacillus rhamnosus* (CCM 1828) and *Candida tropicalis* (CCM 8223). Antimicrobial activity was tested by disc diffusion method.

Key Words: microorganisms, ethanolic herbal extract

INTRODUCTION

Human population growth with its effects on the environment over the past million years has resulted in the emergence of infectious diseases. The discovery of antibiotics during the 20th century coupled with significant advances in antimicrobial drug development improved human health through improved treatment of infections. Even though pharmacological industries have produced a number of new antibiotics in the last years, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agent (Cohen 1992, Aminov 2010). The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, developing research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural (Nascimento et al. 2000). The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. This has significantly limited the efficacy of antibiotics, warranting alternative strategies to combat microbial infections. Bacterial illnesses are orchestrated by means of an array of virulence factors that facilitate various aspects of their pathophysiology critical for disease in the host (Furuya, Lowy 2006, Falkow 1991) These include adhesins and membrane proteins that mediate bacterial attachment, colonization, and invasion of host cells. In addition, microbial toxins cause host tissue damage, and bacterial cell wall components such as capsular polysaccharide confer resistance against host immune system (Wu et al. 2008).

Hence, more studies pertaining to the use of plants as therapeutic agents. The objective of this study was to evaluate the potential of plant extracts on standard microorganism strains.

MATERIAL AND METHODS

In this study, antimicrobial activity of herbal ethanolic extracts was tested by disc diffusion method on the following microorganisms: *Escherichia coli* (CCM 7929), *Enterococcus faecalis* (CCM 4224), *Lactobacillus rhamnosus* (CCM 1828) and *Candida tropicalis* (CCM 8223). These microorganisms were used as a pure cultures from Czech collection of microorganisms. Suspensions of density 1 McF were prepared from 24 hours culture of each bacterium. Herbal ethanolic extracts were prepared from dry and mashed plants *Cannabis sativa L.*, *Silybum marianum* and *Hippophae*

rhamnoides. Herbs were weight out and mixed with 50% ethanol. Our extracts had concentration of 50%, 25% and 10%. Suspensions of bacterium *Escherichia coli*, *Enterococcus faecalis* and *Candida tropicalis* was inoculated on Petri dishes with PCA agar (Biokar diagnostics, France). Suspension of *Lactobacillus rhamnosus* was inoculated on Petri dishes with MRS agar (Biokar diagnostics, France). Sterile paper discs of 9 mm diameter were impregnated with 30 µl of ethanolic extract and placed onto a medium with inoculated bacterium. On each Petri dish, three discs were placed. All variants with bacteria and extracts were performed in triplicate. Prepared Petri dishes were placed in a thermostat at 30°C. Zones of inhibition were twice evaluated by a ruler, after 24 hours and 48 hours. Pure culture of each microorganism were used as control tools.

RESULTS AND DISCUSSION

The average values of diameters of zones of inhibition in mm are stated in Table 1 (after 24 hours) and Table 2 (after 48 hours). If diameter was 9.00 mm, the extract did not exhibit any antimicrobial activity, because 9.00 mm is the diameter of used paper disc.

Table 1 Diameters of inhibitory zones after 24 hours in mm

Microorganism	*Hippophae rhamnoides*			*Silybum marianum*			*Cannabis sativa* L.		
	50%	25%	10%	50%	25%	10%	50%	25%	10%
Escherichia coli	9 9 9	9 9 9	9 9 9	9 9 9	9 9 9	10 11 10	11 10 9	9 9 10	9 10 10
Enterococcus faecalis	9 9 9	9 9 9	9 9 9	10 10 15	11 9 9	12 10 12	9 9 9	9 9 9	9 9 9
Candida tropicalis	9 9 9	9 9 9	9 9 9	9 9 9	9 9 9	9 9 9	9 9 9	9 9 9	9 9 9
Lactobacillus rhamnosus	9 9 9	9 9 9	9 9 9	9 9 9	10 9 9	10 9 9	9 9 9	9 9 9	9 9 9

After 24 hours of incubation is the most effective *Silybum marianum* extract. It is very effective against *Enterococcus faecalis* in each concentration. The inhibitory effect of *Silybum marianum* is observed in 10% concentration against *Escherichia coli* and *Lactobacillus rhamnosus.* Quite effective is *Cannabis sativa L.´*s extract also. Antimicrobial effect against *Escherichia coli* (each of concentration) was demonstrated. The other strains were resistant. Extract from *Hippophae rhamnoides* was evaluated as inefficient. After 48 hours of incubation was confirmed trend, that most effective is extract from *Silybum marianum*. Some of inhibitory zones were smaller than in 1st observation, but this extract is still most effective. We determined inhibitory effect against all of microorganisms: *Enterococcus faecalis* and *Lactobacillus rhamnosus* (in all concentration), *Escherichia coli* (10%), *Candida tropicalis* (25%). As the 2nd more effective ethanolic extract was shown *Hippophae rhamnoides*. It was effective against *Lactobacillus rhamnosus* in all concentration. Against *Candida tropicalis* was effective in higher concentration (50%). Medium concentration (25%) of this herb was efficient against *Enterococcus faecalis*. *Escherichia coli* was resistant against *Hippophae rhamnoides*. The less efficient was *Cannabis sativa L.* extract. It was effective against *Lactobacillus rhamnosus* in all concentration. One zone of inhibitory (12 mm) was observed in 50% concentration by *Escherichia coli. Enterococcus faecalis* and *Candida tropicalis* were resistant in all concentration.

Table 2 Diameters of inhibitory zones after 48 hours in mm

Microorganism	*Hippophae rhamnoides*			*Silybum marianum*			*Cannabis sativa L.*		
	50%	25%	10%	50%	25%	10%	50%	25%	10%
Escherichia coli	9 9 9	9 9 9	9 9 9	9 9 9	9 9 9	10 11 9	9 10 9	9 9 9	9 9 9
Enterococcus faecalis	9 9 9	10 9 9	9 9 9	10 11 10	10 9 9	12 10 11	9 9 9	9 9 9	9 9 9
Candida tropicalis	13 9 9	9 9 9	9 9 9	10 9 9	9 9 9	9 9 9	9 9 9	9 9 9	9 9 9
Lactobacillus rhamnosus	14 10 10	10 11 10	11 11 9	10 10 11	10 12 9	10 11 12	12 14 11	10 10 10	10 10 9

Anecdotal evidence and the traditional use of plants as medicines provide the basis for indicating which essential oils and plant extracts may be useful for medical conditions. Historically, many plant oils and extracts have been used as topical antiseptics, or have been reported to have antimicrobial properties. According to Nissen et al. (2010) *Cannabis sativa L.* exhibited good antimicrobial activities expressed as minimum inhibitory concentrations (2.00% v/v) against gram-positive and gram-negative bacterium. The present results show promising inhibitory activities of *Cannabis sativa L.* against gram-positive opportunistic/pathogens such as *Clostridium* spp. and *Enterococcus* spp. (Sturm et al. 1980, McFarland 2006). The antimicrobial effect of chive against *Escherichia coli* and yeast (*Pichia membranaefaciens* CCRC 20859) has been also reported (Mau et al. 2001). According to Dostalová et al. (2014) parsley in concentration 1:10 and 1:15 expressed strong antimicrobial activity against *Escherichia coli*. Extracts of *Acacia nilotica*, *Cinnamum zeylanicum* and *Syzygium aromaticum* showed the most potent activity against all of the microorganisms studied. *Enterococcus faecalis* and *Escherichia coli* strains were found to be sensitive to extracts of *Acacia nilotica, Cinnamum zeylanicum* and *Syzygium aromaticum* (Khan et al. 2009). According to Parekh and Chanda (2006) none of the extracts except the ethanolic extract of *Launaea procumbens Roxb.* exhibited anticandidal activity against *Candida tropicalis. Candida* sp. was resistant to the extract of *Convolvus althaeoides*, it was affected by extract of *Convolvus arvensis* at extract amounts (200 and 150 $mg.ml^{-1}$). This could be due to the genetic variations between the two species or higher concentration of extract need to be used. The lowest concentration (50 $mg.ml^{-1}$) of *Anthemis pseudocotula* and *Artemisia heba-alba* showed no antimicrobial activity against the growth of *Candida* sp (Hassawi, Kharma 2006). It has been documented that garlic extracts exert a differential inhibition between beneficial intestinal microflora and potentially harmful enterobacteria (Rees et al. 1993). Inhibition observed in *Escherichia coli* was more than 10 times greater than that seen in *Lactobacillus* sp. for the same garlic dose (Skyrme 1997).

CONCLUSION

Some herbs tested in our experiment could be used for animal treatment or as a food or forage preservating. Using of herbs is very important by medicine preparing or for food lifetime extension, because microorganisms cannot develop resistance to their effective compounds. In the future, we plan to test the other herbs against another microorganisms strain.

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