SCREENING OF HERBAL ESSENTIAL OILS' AND ALCOHOLIC EXTRACTIONS' ACTIVITIES ON OEDEMA DISEASE ASSOCIATED *E. COLI* STRAINS

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Summary

The antibacterial efficacy of the ethanol extracts and essential oils of *Thymus vulgaris L.*, *Calendula officinalis L.*, *Salvia officinalis*, and *Hippophae rhamnoides L* was studied *in vitro* against swine strains of *Escherichia coli*. A broth microdilution method was performed in order to determine the antimicrobial potential of these plants and to obtain data regarding the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). No antimicrobial activity was observed when the ethanol extracts were tested (MIC and MBC>4%), whereas two of the essential oils, those obtained from *Thymus vulgaris L* and *Salvia officinalis*, showed strong antimicrobial activity against all isolates, at a concentration that ranged from 0.125 to 1% (v/v). Essential oils of *Thymus vulgaris L* were significantly bactericidal at a minimal concentration of 0.125% (v/v), while *Salvia officinalis* essential oils were less efficient, when compared to *Thymus vulgaris L* ones (MIC and MBC 1%). *Hippophae rhamnoides L.* and *Calendula officinalis L.* essential oils showed no antimicrobial properties.

Our results showed that *Thymus vulgaris L* and *Salvia officinalis* essential oils could be considered as natural antimicrobial agents.

The continuous development of antimicrobial resistance represents a worldwide major concern for both human and veterinary medicine (Delamare *et al.* 2007; Negi *et al.*, 2005). As an alternative strategy to overcome the emerging antimicrobial resistance, herbal preparations are being investigated for their antimicrobial activity. Therefore, nowadays, the growing interest in the antimicrobial screening of natural compounds, such as different kinds of extracts and essential oils from plants for the discovery of new antimicrobial agents is becoming quite obvious.

The aim of this *in vitro* study was to evaluate and compare antimicrobial properties of essential oils and ethanol extracts isolated from *Thymus vulgaris L.*, *Calendula officinalis L.*, *Salvia officinalis*, and *Hippophae rhamnoides L* against porcine strains of *Escherichia coli*. Minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) were determined performing a broth microdilution method.

Materials and methods

Plant extracts: Essential oils and ethanol extracts of: thyme (*Thymus vulgaris L.*, Lamiaceae), marigold (*Calendula officinalis L.*, Asteraceae), common sage (*Salvia officinalis*, Lamiaceae) and common seabuckthorn (*Hippophae rhamnoides L*, Elaeagnaceae) were purchased from commercial sources.

Bacterial strains: reference strain *E. coli* ATCC 10536 and four strains isolated from clinical cases of swine oedema disease. All these strains showed total antibiotic resistance to tetracycline, cephalotin, novobiocin, erythromycin, oxacillin, and three strains formed resistant colonies to enrofloxacin, cephtazidime and trimethoprim-sulphamethoxazole. Bacterial isolates included in this study were cultured overnight at 37◦C on Mueller Hinton agar (Merck). Colonies collected from each twenty-four hours bacterial culture were diluted in sterile saline and the optical density was adjusted according to the tube 0.5 of McFarland' scale in order to prepare a standardized inoculum (1.5×10^8cfu/ml).

Antimicrobial assay: The alcoholic extracts and the essential oils of *Thymus vulgaris L.*, *Calendula officinalis L.*, *Salvia officinalis*, and *Hippophae rhamnoides L* were tested against the five *E. coli* strains. A broth microdilution method previously established by Carson *et al.* (1995) was selected in order to evaluate the antimicrobial potential of these plants and to determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Using Mueller Hinton broth (Merck) supplemented with Tween 20 (0.5% (v/v)), series of twofold dilutions of each essential oil and ethanol extracts were performed in sterile 96-well microtiter plates. 100 µl of each dilution, ranging from 4% to 0.125% (v/v), were mixed with an equal volume of bacterial suspension. Positive and negative growth controls were prepared. Ethanol and Tween 20' antimicrobial activity were also screened. The plates were incubated for 24h, at 37◦C, under normal atmospheric conditions. The MIC was defined as the lowest concentration (highest dilution) of herbal extracts or essential oils that inhibited the visible growth (no turbidity), when compared to the control. Afterwards, 10µl of each well were transferred to Mueller Hinton agar plates and incubated for 24h, at 37◦C. The lowest concentration associated with no visible growth of bacteria on the agar plates was considered the MBC. All dilutions were performed in duplicate.

Results and discussions

In the present study, *E. coli* strains were used in order to evaluate the antimicrobial potential of four plants: *Thymus vulgaris L.*, *Calendula officinalis L.*, *Salvia officinalis*, and *Hippophae rhamnoides L.* The MICs and MBCs values for their ethanol extracts and essential oils, determined using a broth microdilution method, are presented as average values in tables I and II.

This study revealed significant differences in the herbal preparations tested. The level of bacterial growth inhibition induced by plant materials, as

determined by the broth microdilution assay, proved to be dependent mostly on herbal source and type of extract, and then on bacterial strain

As shown in tables I and II, regardless of the extract type – alcoholic or essential oils-, all bacterial isolates were resistant to *Calendula officinalis L.* and *Hippophae rhamnoides L*, although the antimicrobial properties of these plants were recorded in literature (Negi *et al.* 2005; Nohynek *et al.* 2006).

Furthermore, the results of the antibacterial screening indicated that none of the four ethanol extracts were able to inhibit the growth of *E. coli* porcine strains (MIC and MBC >4%).

Table I

Minimum inhibitory concentrations (MICs) % v/v of essential oils and alcoholic extracts

	Salvia officinalis		***Thymus vulgaris***		***Calendula officinalis***		***Hippophae rhamnoides***	
	Ethanol extract	***Essential oils***	***Ethanol extract***	***Essential oils***	***Ethanol extract***	***Essential oils***	***Ethanol extract***	***Essential oils***
t1	> 4	0.125	> 4	0.125	> 4	> 4	> 4	> 4
t2	> 4	1	> 4	0.5	> 4	> 4	> 4	> 4
t3	> 4	1	> 4	0.125	> 4	> 4	> 4	> 4
t4	> 4	1	> 4	0.5	> 4	> 4	> 4	> 4
t5	> 4	0.125	> 4	0.125	> 4	> 4	> 4	> 4

Table II

Minimum bactericidal concentrations (MBCs) % v/v of essential oils and alcoholic extracts

	Salvia officinalis		*Thymus vulgaris*		*Calendula officinalis*		*Hippophae rhamnoides*	
	Ethanol extract	***Essential oils***	***Ethanol extract***	***Essential oils***	***Ethanol extract***	***Essential oils***	***Ethanol extract***	***Essential oils***
t1	> 4	0.125	> 4	0.125	> 4	> 4	> 4	> 4
t2	> 4	1	> 4	0.5	> 4	> 4	> 4	> 4
t3	> 4	1	> 4	0.125	> 4	> 4	> 4	> 4
t4	> 4	1	> 4	0.5	> 4	> 4	> 4	> 4
t5	> 4	0.125	> 4	0.125	> 4	> 4	> 4	> 4

In contrast to these data, several researchers reported that *Thymus vulgaris L* alcoholic extracts displayed antimicrobial effect against *E. coli* strains and other Gram-negative bacteria, due to their complex chemical composition: thymol, carvacrol, borneol, nerol, thymolquinone, p-cymene, gamma-terpinene, (Nohynek *et al.* 2006; Faleiro *et al.* 2003; Horváth *et al.* 2002).

The essential oils usually exhibit a stronger antimicrobial activity than alcoholic extracts (Burt and Reinders, 2003; Echeverrigaray, 2007). This discrepancy in exerting the antibacterial potential may be caused by a variation that concerns the chemical composition as this one is determined at a greater extent by the distillation and extraction technique or by the different geographical chemotype.

The antibacterial activity of the tested essential oils seemed to be strain-dependent, as the MICs and MBCs for t1 and t5 were lower, when compared to those obtained for t2, t3 and t4. Strain variation can be linked to intrinsic strain susceptibility towards the herbal preparations screened.

The bacteriostatic (MICs) and bactericidal (MBCs) values obtained performing the microdilution protocol were well connected (tables I and II). These values suggested that *Thymus vulgaris L* and *Salvia officinalis* essential oils posessed bacteriostatic and bactericidal effects at similar concentrations.

Conclusions

Thymus vulgaris L and *Salvia officinalis* essential oils demonstrated a strong antimicrobial efficacy against all bacterial strains while *Calendula officinalis*

L. and *Hippophae rhamnoides L* did not inhibit the bacterial growth for the tested concentrations (MICs and MBCs > 4% (v/v)). Based on these results, we could conclude that only the *Thymus vulgaris L* and *Salvia officinalis* were effective against all tested microorganisms. These two essential oils possessed promising antibacterial (bacteriostatic and bactericidal) properties against porcine *E coli* strains. The MBCs and MICs ranged from 0.125 to 0.5% (v/v) for *Thymus vulgaris L,* as for *Salvia officinalis* were 0.125 to 1% (v/v).

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